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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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Resistant plants and uses thereof

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Resistant plants and uses thereof

The present invention relates to a novel method for increasing the resitance of a plant, in particular of a Solanaceae, preferably of potato and tomato, to plant pathogens of the phylum Oomyceta comprising increasing the activity of the polypeptid of the present invention. The invention further relates to polynucleotides and vectors comprising these polynucleotdes. The invention furthermore relates to corresponding vectors, cells transgenic plants and transgenic propagation material derived from them, methods to produce them and to their use for the production of foodstuffs, feeding stuffs, seed, pharmaceuticals or fine chemicals.

The aim of plant biotechnology work is the generation of plants with advantageous novel properties, for example for increasing agricultural productivity, increasing the quality in the case of foodstuffs, or for producing specific chemicals or pharmaceuticals (Dunwell JM (2000) J Exp Bot 51 Spec No:487-96). The plant's natural defense mechanisms against pathogens are frequently insufficient. Fungal diseases alone result in annual yield losses of many billions of US$. The introduction of foreign genes from plants, animals or microbial sources can increase the defenses. Examples are the protection of tobacco against feeding damage by insects by expressing *Bacillus thuringiensis* endotoxins under the control of the 35S CaMV promoter (Vaeck et al. (1987) Nature 328:33-37) or the protection of tobacco against fungal infection by expressing a bean chitinase under the control of the CaMV promoter (Broglie et al. (1991) Science 254:1194-1197). However, most of the approaches described only offer resistance to a single pathogen or a narrow spectrum of pathogens.

Despite the notorious Irish potato famine of the mid-19th century, late blight still continues to be one of the most devastating of all diseases in crop plants. Late blight is caused by the oomycete fungus *Phytophthora infestans*, a specialised pathogen, primarily causing disease on the foliage and fruits of a range of Solanaceae species, especially potato and tomato. The fungus was first observed in Mexico and for several reasons Mexico is believed to be the centre of origin of the fungus. Both of the mating types A1 and A2 are permanently present in for example the Toluca area. Also, *P. infestans* is reported on native *Solanum* species in remote areas of Mexico. Furthermore, many species of tuber bearing Solanum with a high level of resistance to late blight are found in Mexico. Prevailing measures to prevent crop failures or reduced yields imply the application of fungicides that prevent or cure an infection by *P. infestans*. Instead of the massive use of chemical pesticides an alternative approach for controlling late blight could be advantageous: the use of cultivars, which harbour partial or complete resistance to late blight. To obtain late blight resistance, breeders have in the past focussed on the introgression of dominant R genes from *Solanum demissum*, a wild potato species indigenous to Mexico. Eleven such R genes have been

identified, several of which have been mapped to specific loci on the genetic map of potato (reviewed in Gebhardt and Valkonen, 2001) and recently the R1 gene has been cloned. R^1 and R^2 are located on chromosomes 5 and 4, respectively. R^3, R^6 and R^7 are located on chromosome 11. Unknown R genes conferring race specific resistance to late blight have also been described in S. tuberosum ssp. Andigena and S. berthaultii and pennapisecpum. The resistance induced by these R-genes was (nearly) complete but appeared not to be durable in any case. Because of the high level of resistance and ease of transfer, many cultivars contain S. demissum derived resistance. Unfortunately, S. demissum derived race specific resistance, although nearly complete, is not durable. Once newly bred potato cultivars were grown on larger scale in commercial fields, new virulences emerged in *P. infestans*, which rendered the pathogen able to overcome the introgressed resistance. More durable field resistance to late blight, often quantitative in nature and presumed to be race non-specific, can be found in several Mexican and Central and South American *Solanum* species. However this type of resistance is difficult to transfer into potato cultivars through crossing and phenotypic selection.

Diploid *S. bulbocastanum* from Mexico and Guatemala is one of the tuber bearing species that is long known for its high levels of resistance to late blight. Unfortunately, classic transfer of resistance from wild *Solanum* species to cultivated potato is frequently prevented due to differences in ploidy and Endosperm Balance Number (EBN). Despite these problems, introgression of the *S. bulbocastanum* resistance trait has been successful. Recently, somatic hybrids of *S. bulbocastanum* and *S. tuberosum* and backcrossed germplasm were found to be highly resistant to late blight, even under extreme disease pressure (Helgeson *et al.*, 1998). Some of these hybrids and backcrossed germplasm were found to be highly resistant to late blight, even under extreme disease pressure. Despite reports of suppression of recombination, resistance in the backcrossed material appeared to be on chromosome 8 within an approximately 6 cM interval between the RFLP markers CP53 and CT64. A CAPS marker derived from the tomato RFLP probe CT88 cosegregated with resistance.

Accordingly, in the recent years the development of plants resistant to pathogens of the phylum Oomycota forged ahead. However, 40 years of intense and continuous research and breeding efforts with this germplasm has still not resulted in market introduction of resistant cultivars. The prevailing number of genes identified in the recent years confers merely race specific resistance. Further, the achieved resistance was not durable. In addition, the application of crop protectants is widely considered to be a burden for the environment. Thus, in several Western countries, legislation becomes more restrictive and partly prohibitive to the application of specific fungicides, making chemical control of the disease more difficult. Further, chemical control is expensive. Finally, another restriction is the development of resistance by the fungus to specific

fungicides such as metalaxyl, which has been reported from many countries in the world.

Accordingly, the problem underlying the present invention is to provide novel means and methods for a efficient protection of plants against late blight and related diseases.

The solution of the technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a method for generating or increasing the resistance of a plant to plant pathogen of the phylum Oomycota comprising increasing the activity of Rpi-blb2 protein in the plant or a tissue, organ or cell of the plant or a part thereof.

Rpi-blb2 is a LZ-NBS-LRR type of R gene and shows sequence homology to the tomato gene Mi-1, that confers resistance to three species of root knot nematodes (*Meloidogyne* spp.) as well as to the potato aphid *Macrosiphum euphorbiae* (Vos *et al.*, 1998; Rossi *et al.*, 1998; Milligan *et al.*, 1998) and to both B- and Q-biotypes of whitefly *Bemisia tabaci* (Nombela *et al.*, 2003). As was found for *Rpi-blb*, *Rpi-blb2* also confers full resistance to a range of *P. infestans* isolates carrying multiple virulence factors and race-specificity has not yet been demonstrated.

By the term "generating" or "increasing" or "stimulating" "the resistance of a plant" is meant that the resistance of a plant or a part thereof is increased in comparison to a reference.

"Conferring", "existing", "generating", "stimulating" or "increasing" a pathogen resistance means that the defense mechanisms of a specific plant species or variety is increasingly resistant to one or more pathogens due to the use of the method according to the invention in comparison with the wild type of the plant, to which the method according to the invention has not been applied, under otherwise identical conditions (such as, for example, climatic conditions, growing conditions, pathogen species and the like). The increased resistance manifests itself preferably in a reduced manifestation of the disease symptoms, disease symptoms comprising - in addition to the abovementioned adverse effects - for example also the penetration efficiency of a pathogen into the plant or plant cells or the proliferation efficiency in or on the same. In this context, the disease symptoms are preferably reduced by at least 10% or at least 20%, especially preferably by at least 40% or 60%, very especially preferably by at least 70% or 80% and most preferably by at least 90% or 95%. Further bei the term "increased" it is hereby meant that the resistance or activity is higher than in a reference or the resistance or activity is generated de novo, if no resistance could be observed in the reference. The stimulation of a resistance or activity is also under the scope of the term "increased resistance" or "increased activity". The activation of the

a gene, i.e. the promoter, can be stimulated, e.g. by applying chemicals or by biotic stress, e.g. by transfection with P. infestans, conferring resistance to a pathogen. In the following, the term "increasing" also comprises the term "stimulating".

"Pathogen resistance" denotes the reduction or weakening of disease symptoms of a plant following infection by a pathogen. The symptoms can be manifold, but preferably encompass those which directly or indirectly have an adverse effect on the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff, or else which make sowing, planting, harvesting or processing of the crop difficult.

"Pathogen" within the scope of the invention means by way of example but not by limitation viruses or viroids, bacteria, fungi, animal pests such as, for example, insects or nematodes.

The term "Rpi-blb2 protein" relates to a protein or polypeptid which expression in a plant or a part confers resistance of the plant or a part of the plant to one of pathogens described herein in comparison to a non-resistant strain.
The plant or a tissue, organ or cell of the plant or a part thereof comprising increased activity of Rpi-blb2 protein is less susceptible to an infection by a pathogen, in particular to pathogen of the phylum Oomyceta preferably to P. infestans, than a plant or a part thereof which has the identical genetic background but not the genetic elements necessary to allow an expression of Rpi-blb2 (herein named as "wild type" or "reference"). Assays for the testing of the resistance of a plant or a part thereof are well known to a person skilled in the art. The resistance to P. infestans can be defined as Sporulation index according to Flier, 2001. Flier describes the sporulation index as level of sporulation per 1 cm^2. Thus, a reduction of sporulation per 1 cm^2 of 20% compared to a wild typ is herein defined as resistance. In the examples illustrating the present invention, the sporulation index was defined as level of sporulation per lesion. Thus, by the term "resistance" is alternatively meant a reduction of sporulation per lesion of 20% compared to a wild type. The later definition is preferred. In preferred embodiments the sporulation in an assay is reduced by 30%, more preferred is a reduction of 50%, even more preferred are 70%, even more preferred are more than 80%, more preferred are 85% and 90%. Most preferred is 95% or more.

Accordingly, in the present invention by "activity" of a Rpi-blb2 protein is meant, that the protein expression confers said reduction in the sporulation index. Further, it was observed, that a typical response for plants containing Rpi-blb2 to a P. infestans infection is the presence of small lesions, without any clear sporulation, at the end of the growing season. Thus, in one embodiment, the activity of Rpi-blb2 is defined as the presence of small lesions without any clear sporulation.

The term "reference" relates to an organism which is genetically identical to the Organism of the present invention or a part thereof but a expression of Rpi-blb2 can not be observed as there is a little difference in the genom, proteom or metabolom. Therefore the reference is a plant or a part thereof which does not encode Rpi-blb2 or does not transcribe Rpi-blb2 gene or does not translate a Rpi-blb2 mRNA or does not provide any modification for creating an active mRNA or polypeptide. Whether two plants are genetically identical can be tested with assays known to a person skilled in the art, e.g. via fingerprint analysis, e.g. as described in Roldan-Ruiz. Theor. Appl. Genet., 2001. 1138-1150. In order to increase the resistance the reference is susceptible to the infection with a plant pathogen of said group.
Preferably, the reference is a clone of that organism in which the polynucleotide of the invention or an activator of the expression of said polynucleotide or a derivate of said polynucleotide or a polypeptide of the present invention or a corresponding vector has been introduced. For example, a preferred reference is the strain before it has been transfected or transformed with the polynucleotid or vector of the invention. If the strain can not be identified it is state of the art to cleave the elements which mediate Rpi-blb2 expression out of the genome of the plant or to inhibit the expression of the Rpi-blb2 protein by other methods. Such a strain can than be compared with the resistant, Rpi-blb2 expressing strain.

The term "plant" as used herein refers to all genera and species of higher and lower plants of the Plant Kingdom. The term includes the mature plants, seed, shoots and seedlings and their derived parts, propagation material, plant organs, tissue, protoplasts, callus and other cultures, for example cell cultures, and any other type of plant cell grouping to give functional or structural units. Mature plants refers to plants at any desired developmental stage beyond that of the seedling. Seedling refers to a young immature plant at an early developmental stage. "Plant" encompasses all annual and perennial monocotyledonous and dicotyledonous plants. Preferred within the scope of the invention are those plants which are employed as foodstuffs or feeding stuffs, very especially preferaby monocotyledonous genera and species like the above-described cereal species.

As known to a person skilled in the art, the method of the present invention comprises further selecting those plants in which, as opposed or as compared to the original plant, the resistance to at least one said pathogen exists or is increased.
"Selection" with regard to plants in which - as opposed or as compared to the original plant - resistance to at least one pathogen exists or is increased means all those methods which are suitable for recognizing an existing or increased resistance to pathogens. These may be symptoms of pathogen infection but may also comprise the herein

described symptoms which relate to the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff and the like.

Accordingly, in one embodiment of the method of present invention the Rpi-blb2 protein is encoded by a polynucleotide comprising a nucleic acid molecule selected from the group consisting of:

a) nucleic acid molecules encoding at least the mature form of the polypeptide depicted in SEQ ID NO: 2 or 4;

b) nucleic acid molecules comprising the coding sequence as depicted in SEQ ID NO: 1 or 3, or 5 or 6 encoding at least the mature form of the polypeptide;

c) nucleic acid molecules the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b);

d) nucleic acid molecules encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) to (c) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) to (c);

e) nucleic acid molecules encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a) or (b);

f) nucleic acid molecules comprising a fragment or a epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);

g) nucleic acid molecules comprising a polynucleotide having a sequence of a nucleic acid molecule amplified from a nucleic acid library using the primers as listed in Tab 3b, in particular ARF1F and ARF1R;

h) nucleic acid molecules encoding a fragment beginning with amino acid: 1, 30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1276, 1000, 500, 300, 200, 50, or 1 of a polypeptide encoded by any one of (a) to (g);

i) nucleic acid molecules comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (d);

j) nucleic acid molecules encoding a polypeptide being recognized by a monoclonal antibody that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (h);

k) nucleic acid molecules obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (j) or of a fragment thereof of at least 15, preferable 30, 60, 100 or more nucleotides; and

l) nucleic acid molecules the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);

or the complementary strand of any one of (a) to (l);

or expressing a polypeptide encoded by a segment or linkage group 6 of Solanum bulbocastanum which co-segregates with a marker selected from table 3A and which mediates resistance to pathogens, in particular to pathogens selected from the group consisting of phylum Oomyceta;

and whereby the polynucleotide has not the sequence of Mi1.1 or Mi1.2 as depicted in Seq. ID NO.: 7 and 9 or encoding a Mi1.1 or Mi1.2 protein as depicted in Seq. ID NO.: 8 and 10.

Accordingly, in one embodiment the present invention relates to the method of the present invention, wherein the Rpi-blb2 protein is encoded by the polynucleotide of the present invention.

On basis of a BLASTX search the genes with the highest homology identified were the Mi1.1- and Mi1.2-genes. Both genes have a high identity to the sequence depicted in Seq. ID NO.: 1 or 3 or 5 or 6 but do not confer restistance to the plant pathogen of the phylum Oomyceta. Therefore the activity of Mi1.1 and Mi1.2 is an other activity as the activity of the polypeptid of the present invention. The sequence of Mi1.1 and Mi1.2 is herein shown in Seq. ID NO.: 7 to 10. Further, the application EP 401764.4 relates to the Mi-genes. The sequence of prior art Mi1.1- and Mi1.2-genes is excluded from the polynucleotide of the present invention, in particular Seq. ID NO.: 7 and 9 are excluded. Also included may be polynucelotide sequences encoding the polypeptide of Seq. ID NO.: 8 or 10. Thus, in an embodiment also sequences encoding the Mi1.1 and Mi1.2 protein are excluded. Proteins with a lower homology to the polypeptide encoded by the polynuncleotide of the present invention are Hero Resistance proteins 1 and 2 (Genbank AccNo.: gi26190252 and gi26190254), Tospovirus resistance proteins A, B, C, D and E [Genbank AccNos.:gi15418709, gi15418710, gi15418712, gi15418713, gi15418714]; R1 [Genbank AccNo.: gi17432423] and Prf [Genbank AccNo.: gi8547237] which sequences or encoded sequences are as well excluded from the sequences of the present invention.

The terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid molecule(s)" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule.

Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, "caps" substitution of one

or more of the naturally occurring nucleotides with an analog. Preferably, the DNA sequence of the invention comprises a coding sequence encoding the herein defined polypeptide.

A "coding sequence" is a nucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

By "hybridizing" it is meant that such nucleic acid molecules hybridize under conventional hybridization conditions, preferably under stringent conditions such as described by, e.g., Sambrook (Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)). An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for one hour. Alternatively, an exemplary stringent hybridization condition is in 50 % formamide, 4XSSC at 42°C. Further, the conditions during the wash step can be selected from the range of conditions delimited by low-stringency conditions (approximately 2X SSC at 50°C) and high-stringency conditions (approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3M sodium citrate, 3M NaCl, pH 7.0). In addition, the temperature during the wash step can be raised from low-stringency conditions at room temperature, approximately 22°C, to higher-stringency conditions at approximately 65°C. Both of the parameters salt concentration and temperature can be varied simultaneously, or else one of the two parameters can be kept constant while only the other is varied. Denaturants, for example formamide or SDS, may also be employed during the hybridization. In the presence of 50% formamide, hybridization is preferably effected at 42°C. Some examples of conditions for hybridization and wash step are shown hereinbelow:

(1) Hybridization conditions can be selected, for example, from the following conditions:

a) 4X SSC at 65°C,

b) 6X SSC at 45°C,

c) 6X SSC, 100 mg/ml denatured fragmented fish sperm DNA at 68°C,

d) 6X SSC, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA at 68°C,

e) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA, 50% formamide at 42°C,

f) 50% formamide, 4X SSC at 42°C,

g) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrate at 42°C.

h) 2X or 4X SSC at 50°C (low-stringency condition). or

i) 30 to 40% formamide, 2X or 4X SSC at 42°C (low-stringency condition).

(2) Wash steps can be selected, for example, from the following conditions:

a) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.

b) 0.1X SSC at 65°C.

c) 0.1X SSC, 0.5 % SDS at 68°C.

d) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C.

e) 0.2X SSC, 0.1% SDS at 42°C.

f) 2X SSC at 65°C (low-stringency condition).

Rpi-blb2 derived from other organisms, may be encoded by other DNA sequences which hybridize to the sequences shown in Seq ID No. 1 or 3 or 5 or 6 under relaxed hybridization conditions and which code on expression for peptides having the activity of Rpi-blb2. Further, some applications have to be performed at low stringency hybridisation conditions, without any consequences for the specificity of the hybridisation. For example, a Southern blot analysis of total DNA could be probed with a polynucleotide of the present invention and washed at low stringency (55°C in 2xSSPE, o,1% SDS). The hybridisation analysis could reveal a simple pattern of only genes encoding Rpi-blb2. A further example of such low-stringent hybridization conditions are 4XSSC at 50°C or hybridization with 30 to 40% formamide at 42°C. Such molecules comprise those which are fragments, analogues or derivatives of Rpi-blb2 of the invention and differ, for example, by way of amino acid and/or nucleotide deletion(s), insertion(s), substitution (s), addition(s) and/or recombination (s) or any other modification(s) known in the art either alone or in combination from the above-described amino acid sequences or their underlying nucleotide sequence(s). However, it is preferred to use high stringency hybridisation conditions.

The term "homology" means that the respective nucleic acid molecules or encoded proteins are functionally and/or structurally equivalent. The nucleic acid molecules that are homologous to the nucleic acid molecules described above and that are derivatives of said nucleic acid molecules are, for example, variations of said nucleic acid molecules which represent modifications having the same biological function, in particular encoding proteins with the same or substantially the same biological function. They may be naturally occurring variations, such as sequences from other plant varieties or species, or mutations. These mutations may occur naturally or may be obtained by mutagenesis techniques. The allelic variations may be naturally occurring allelic vari-

ants as well as synthetically produced or genetically engineered variants. Structurally equivalents can, for example, identified by testing the binding of said polypeptide to antibodies. Structurally equivalent have the similar immunological characteristic, e.g. comprise similar epitopes.

The terms "fragment", "fragment of a sequence" or "part of a sequence" mean a truncated sequence of the original sequence referred to. The truncated sequence (nucleic acid or protein sequence) can vary widely in length; the minimum size being a sequence of sufficient size to provide a sequence with at least a comparable function and/or activity of the original sequence referred to, while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired activity and/or function(s) of the original sequence.

Typically, the truncated amino acid sequence will range from about 5 to about 1260 amino acids in length. More typically, however, the sequence will be a maximum of about 1000 amino acids in length, preferably a maximum of about 500 or 100 amino acids. It is usually desirable to select sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

The term "epitope" relates to specific immunoreactive sites within an antigen, also known as antigenic determinates. These epitopes can be a linear array of monomers in a polymeric composition – such as amino acids in a protein – or consist of or comprise a more complex secondary or tertiary structure. Those of skill will recognize that all immunogens (i.e., substances capable of eliciting an immune response) are antigens; however, some antigen, such as haptens, are not immunogens but may be made immunogenic by coupling to a carrier molecule. The term "antigen" includes references to a substance to which an antibody can be generated and/or to which the antibody is specifically immunoreactive. In one embodiment the present invention relates to a epitope of Rpi-blb2.

The term "one or several amino acids" relates to at least one amino acid but not more than that number of amino acids which would result in a homology of below 70% identity. Preferably, the identity is more than 75% or 80%, more preferred are 85%, 90% or 95%, even more preferred are 96%, 97%, 98%, or 99% identity.

The terms "polynucleotide" and "nucleic acid molecule" also relate to "isolated" polynucleotides or nucleic acids molecules. An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic

acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the polynucleotide of the present invention can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb or less of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, the polynucleotides of the present invention, in particular an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

Further, the polynucleotide of the invention comprises a nucleic acid molecule which is a complement of one of the nucleotide sequences of above mentioned polynucleotides or a portion thereof. A nucleic acid molecule which is complementary to one of the nucleotide sequences shown in SEQ ID No:1 or 3 or 5 or 6 is one which is sufficiently complementary to one of the nucleotide sequences shown in SEQ ID No: 1 or 3 or 5 or 6 such that it can hybridize to one of the nucleotide sequences shown in SEQ ID No: 1 or 3 or 5 or 6, thereby forming a stable duplex.

The polynucleotide of the invention comprises a nucleotide sequence which is at least about 70%, preferably at least about 75%, more preferably at least about 80%, 90%, or 95%, and even more preferably at least about 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence shown in SEQ ID No: 1 or 3 or 5 or 6, or a portion thereof. The polynucleotide of the invention comprises a nucleotide sequence which hybridizes, preferably hybridizes under stringent conditions as defined herein, to one of the nucleotide sequences shown in SEQ ID No: 1 or 3 or 5 or 6, or a portion thereof.

Moreover, the polynucleotide of the invention can comprise only a portion of the coding region of one of the sequences in SEQ ID No: 1 or 3 or 5 or 6, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of an Rpi-blb2. The nucleotide sequences determined from the cloning of the the present Rpi-blb2 protein encoding gene allows for the generation of probes and primers designed for use in identifying and/or cloning its homologues in other cell types and organisms. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 15 preferably about 20 or 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense strand of one of the sequences set forth, e.g., in SEQ ID No. No: 1 or 3 or 5 or 6, an anti-sense sequence of one of the sequences, e.g., set forth in SEQ ID No.: 1 or 3 or 5 or 6, or naturally occurring mutants thereof. Primers based on a nucleotide of invention can be used in PCR reactions to clone Rpi-blb2 homologues, e.g. as the primers described

In the examples of the present invention, e.g. as shown in tab 3a or 3b, preferably the primers ARF1F and ARF1R are used. A PCR with the primers univ24R and univ14L will result in a fragment of Rpi-blb2 which can be used as described herein. Said primer sets are interchangable. The person skilled in the art knows to combine said primers to result in the desired product, e.g. in a full length clone or a partial sequence. Probes based on the Rpi-blb2 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. The probe can further comprise a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a genomic marker test kit for identifying cells which express an Rpi-blb2, such as by measuring a level of an Rpi-blb2-encoding nucleic acid molecule in a sample of cells, e.g., detecting Rpi-blb2 mRNA levels or determining whether a genomic Rpi-blb2 gene has been mutated or deleted.

The polynucleotide of the invention encodes a polypeptide or portion thereof which includes an amino acid sequence which is sufficiently homologous to the amino acid sequence of SEQ ID No: 2 or 4 such that the protein or portion thereof maintains the ability to participate in resistence to pathogens, in particular a Rpi-blb2 activity as described in the examples in plants. As used herein, the language "sufficiently homologous" refers to proteins or portions thereof which have amino acid sequences which include a minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain as an amino acid residue in one of the sequences of the polypeptide of the present invention, amino acid residues to an amino acid sequence of Seq. ID No.: 2 or 4 such that the protein or portion thereof is able to participate in the resistance of plants to said pathogens. Examples of a Rpi-blb2 activity are described herein. Thus, the function of an Rpi-blb2 protein contributes either directly or indirectly to the resistance to plant pathogens, preferably to the pathogens mentioned herein, more preferred to P. infestans.

The protein is at least about 70%, preferably at least about 75%, and more preferably at least about 80%, 90%, 95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of SEQ ID No: 2 or 4.

Portions of proteins encoded by the polynucleotide of the invention are preferably biologically active.
As mentioned herein, the term "biologically active portion" is intended to include a portion, e.g., a domain/motif, that confers resistance to an oomycete plant pathogene and/or Bemisia tabaci and/or aphids or has an immunological activity such that it is binds to an antibody binding specifially to Rpi-blb2 or it has an activity as set forth in the Examples or as described herein.

Additional nucleic acid fragments encoding biologically active portions of the polypetide of the present invention can be prepared by isolating a portion of one of the sequences in SEQ ID No: 1 or 3 or 5 or 6, expressing the encoded portion of the Rpi-blb2 or peptide (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the protein.

The invention further encompasses polynucleotides that differ from one of the nucleotide sequences shown in SEQ ID No: 1 or 3 or 5 or 6 (and portions thereof) due to degeneracy of the genetic code and thus encode a Rpi-blb2 as that encoded by the sequences shown in SEQ ID No: 2 or 4. Further the polynucleotide of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID No: 2 or 4. In a still further embodiment, the polynucleotide of the invention encodes a full length protein which is substantially homologous to an amino acid sequence of SEQ ID No: 2 or 4.

In addition, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences may exist within a population (e.g., the S. bulbocastanum population). Such genetic polymorphism in the Rpi-blb2 gene may exist among individuals within a population due to natural variation.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an Rpi-blb2, preferably a S. bulbocastanum Rpi-blb2. Such natural variations can typically result in 1-5% variance in the nucleotide sequence of the Rpi-blb2 gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in Rpi-blb2 that are the result of natural variation and that do not alter the functional activity of Rpi-blb2 are intended to be within the scope of the invention.

Polynucleotides corresponding to natural variants and non-S. bulbocastanum homologues of the Rpi-blb2 cDNA of the invention can be isolated based on their homology to S. bulbocastanum Rpi-blb2 polynucleotides disclosed herein using the polynucleotide of the invention, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Accordingly, in another embodiment, an polynucleotide of the invention is at least 20 nucleotides in length. Preferably it hybridizes under stringent conditions to the nucleic acid molecule comprising a nucleotide sequence of the polynucleotide of the present invention, e.g. SEQ ID No: 1 or 3 or 5 or 6. In other embodiments, the nucleic acid is at least 20, 30, 50, 100, 250 or more nucleotides in length. The term "hybridizes under stringent conditions" is defined above and is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 65% identical to each other

typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 75% or 80%, and even more preferably at least about 85%, 90% or 95% or more identical to each other typically remain hybridized to each other. Preferably, polynucleotide of the invention that hybridizes under stringent conditions to a sequence of SEQ ID No: 1 or 3 or 5 or 6 corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). Preferably, the polynucleotide encodes a natural S. bulbocastanum Rpi-blb2.

In addition to naturally-occurring variants of the Rpi-blb2 sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into a nucleotide sequence of the polynucleotide encoding Rpi-blb2, thereby leading to changes in the amino acid sequence of the encoded Rpi-blb2, without altering the functional ability of the Rpi-blb2. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of the polynucleotide encoding Rpi-blb2, e.g. SEQ ID No: 1 or 3 or 5 or 6. A"non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one of the Rpi-blb2 without altering the activity of said Rpi-blb2, whereas an "essential" amino acid residue is required for Rpi-blb2 activity. Other amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having Rpi-blb2 activity) may not be essential for activity and thus are likely to be amenable to alteration without altering Rpi-blb2 activity.

Accordingly, a person skilled in the art knows that the codon usage between organism can differ. Therefore he will adapt the codon usage in the polynucleotide of the present invention to the usage of the organism in which the polynuclestide or polypeptide is expressed.

Accordingly, the invention relates to polynucleotides encoding Rpi-blb2 that contain changes in amino acid residues that are not essential for Rpi-blb2 activity. Such Rpi-blb2s differ in amino acid sequence from a sequence contained in SEQ ID No: 2 or 4 yet retain the Rpi-blb2 activity described herein. The polynucleotide can comprise a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 70% identical to an amino acid sequence of EQ ID No: 2 or 4 and is capable of participation in the resistance to a plant pathogen. Preferably, the protein encoded by the nucleic acid molecule is at least about 70% identical to the sequence in SEQ ID No: 2 or 4. more preferably at least about 75% identical to one of the sequences in SEQ ID No: 2 or 4, even more preferably at least

bly at least about 80%, 90%, 95% homologous to the sequence in SEQ ID No: 2 or 4, and most preferably at least about 96%, 97%, 98%, or 99% identical to the sequence in SEQ ID No: 2 or 4.

To determine the percent homology of two amino acid sequences (e.g., one of the sequences of Seq. ID No.: 2 or 4 and a mutant form thereof) or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence (e.g., one of the sequences of SEQ ID No: 2 or 4) is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence (e.g., a mutant form of the sequence selected), then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = numbers of identical positions/total numbers of positions x 100).

Homology can be calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA; Altschul et al. (1997) Nucleic Acids Res. 25:3389 et seq.), setting the following parameters:

Gap weight:	50	Length weight:	3
Average match:	10	Average mismatch:	0

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 1 at the nucleic acid level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 1 by the above program algorithm with the above parameter set, has at least 80% homology.

Homology between two polypeptides is understood as meaning the identity of the amino acid sequence over in each case the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap weight:	8	Length weight:	2
Average match:	2,912	Average mismatch:	-2,003

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 2 at the protein level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 2 by the above program algorithm with the above parameter set, has at least 80% homology.

In the present application, the homology was determined with the program clustalW which can be found on www.ebi.ac.uk/tools, choose sequence analyses and choose option clustalW (multiple sequence alignments). All options were performed under standard conditions, as follows:
alignment: full; output format: aln w/numbers; output order: aligned; color alignment: no; ktup (word size): def; window length: def; score type: percent; topdiag: def; pairgap: def; matrix: def; gap open: def; end gaps: def; gap extension: def; gap distances: def; cpu mode: single; tree graph/ type: cladogram; tree graph / distances: hide; phylogenetic tree/tree type: none; phylogenetic tree/correct dist.: off; phylogenetic tree/ ignore gaps: off. Therefore a Homology calculation according to clustalW is preferred.

Functional equivalents derived from one of the polypeptides as shown in SEQ ID NO: 2 or 4 according to the invention by substitution, insertion or deletion have at least 70%, preferably at least 80%, by preference at least 90%, especially preferably at least 95%, very especially preferably at least 98%, homology with one of the polypeptides as shown in SEQ ID NO: 2 or 4 according to the invention and are distinguished by essentially the same properties as the polypeptide as shown in SEQ ID NO: 2 or 4.

Functional equivalents derived from the nucleic acid sequence as shown in SEQ ID NO: 1 or 3 or 5 or 6 according to the invention by substitution, insertion or deletion have at least 70%, preferably at least 80%, by preference at least 90%, especially preferably at least 95%, very especially preferably at least 98%, homology with one of the polypeptides as shown in SEQ ID NO: 2 or 4 according to the invention and encode polypeptides having essentially the same properties as the polypeptide as shown in SEQ ID NO: 2 or 4.
"Essentially the same properties" of a functional equivalent is above all understood as meaning conferring a pathogen-resistant phenotype or conferring or increasing the resistance to at least one pathogen while increasing the amount of protein, activity or function of said functional Rpi-blb2 equivalent in a plant or in a tissue, part or cells of the same. The sporulation and lesion phenotyp after infection in combination with said increase of the amount of protein, activity or function of the functional equivalent is furthermore understood as an essential property.

A nucleic acid molecule encoding an Rpi-blb2 homologous to a protein sequence of SEQ ID No: 2 or 4 can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of the polynucleotide of the present invention, in particular of SEQ ID No: 1 or 3 or 5 or 6 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into the sequences of, e.g., SEQ ID No: 1 or 3 or 5 or 6 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Pref-

erably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an Rpi-blb2 is preferably replaced with another amino acid residue from the same family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an Rpi-blb2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for an Rpi-blb2 activity described herein to identify mutants that retain Rpi-blb2 activity. Following mutagenesis of one of the sequences of SEQ ID No: 1 or 3 or 5 or 6, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using, for example, assays described herein (see Examples).

In one embodiment, in the method of present invention the activity of Rpi-blb2 protein and of a further resistance protein is increased.
It is expected, that under field conditions the presence of more than one resistance gene is benefical, in particular genes conferring resistance to the same pathogen. In case a pathogen isolate, e.g. a P. infestans isolate, is present that is able to overcome resistance of one of the R-genes, the other one or more R-gene(s) is/are still functional making it impossible to infect the plant. The present of two undefeated R-genes strongly reduces the chance that a pathogen, in particular a P. infestans isolate, is able to mutate into an isolate that can overcome two or more R-genes.
In the following "resistance polypeptide" or "resistance protein" relates to a polypeptide which (increased) activity will confer resistance to a susceptible strain ("wild type" or "reference"). Accordingly, Rpi-blb2 is a resistance protein as well as e.g. Rpi-blb (or RB). A "further resistance protein" relates to an other resistance protein than the protein of the present invention, whereas the term "resistance protein" comprises the polypeptid of the present invention as well as one or more further resistance protein(s). It is further understood, that the term "and a further resistance protein" relates to one or more further resistance proteins. Thus, the activity of one or more resistance proteins can be increased. Further resistance proteins are described below. However, generally any other known resistance protein can be co-expressed with the polypeptid of the pre-

sent invention or its activity can be increased by any of the methods described herein for Rpi-blb2.

In a preferred embodiment, the further resistance protein comprises a LRR domain and a P-loop.

The cloning and molecular characterisation of over 30 plant disease resistance (*R*) genes conferring resistance to bacteria, fungi, oomycetes, viruses, nematodes, or insects has allowed their classification in structural classes regardless of pathogen specificity (reviewed in Dangl and Jones, 2001). The most abundant class of characterised *R* genes, comprising about 0.5 percent of the genes predicted in the *Arabidopsis* genome, is predicted to encode intracellular proteins that carry leucine-rich repeat (LRR) and nucleotide-binding site (NBS) domains, motifs also found in other receptor and signal transduction proteins. NBS-LRR *R* proteins differ primarily at the N-terminus that either exhibits sequence similarity to the *Drosophila* Toll protein and the mammalian interleukin-1 receptor domain (TIR-NBS-LRR), or code for a coiled-coils structure (CC-NBS-LRR), sometimes in the form of a leucine zipper (LZ-NBS-LRR. Although maybe membrane associated, NBS-LRR proteins are predicted to be cytoplasmic. In contrast, two other classes of *R* proteins that carry LRRs are predicted to span the cell membrane, with an extracellular LRR domain: the LRR-transmembrane (LRR-TM) *Cf* proteins and the LRR-TM-kinase *Xa21* protein. Characterised *R* proteins that lack LRRs are the *Pto* gene from tomato, the *Hs1^{pro-1}* gene from beet, the *mlo* gene from barley, the *Rpw8* genes from *Arabidopsis* and the *Rpg1* gene from barley.

According to the gene-for-gene hypothesis, disease resistance follows perception by plant *R* proteins of pathogen effector molecules with avirulence (*Avr*) function, thereby initiating through some kind of elicitor recognition complex, signal transduction pathways leading to a hypersensitive response (HR). In common with other receptors it is generally considered that NBS-LRR *R* proteins have a modular structure with separate recognition and signalling domains, whereby the LRR is the candidate recognition domain and the N-terminal region including the NBS, the major signalling domain. Functional analysis of recombinant *R* proteins indicates that recognition specificity indeed resides in the LRR. Moreover, the LRR is the most variable region in closely related NBS-LRR proteins and is under selection to diverge. However, evidence is accumulating that LRRs also contribute to signalling through negative regulation involving putative intramolecular interactions. Currently, five *R* genes have been cloned from potato, including two R genes conferring resistance to late blight, and all belong to the CC/LZ-NBS-LRR class of plant *R* genes. While the *S. demissum* derived *R1* gene confers race specific resistance to late blight, the recently cloned *S. bulbocastanum* derived gene *Rpi-blb (or RB)* confers full resistance to a range of *P. infestans* isolates carrying multiple virulence factors and race-specificity has not yet been demonstrated. Furthermore, as described before, progeny plants of somatic hybrids containing *Rpi-blb* were

unaffected by late blight on field experiments in Mexico, where nearly every race of the fungus is found. Through complementation of the susceptible phenotype in cultivated potato and tomato the potential of interspecific transfer of broad-spectrum late blight resistance to cultivated *Solanaceae* from sexually incompatible host species by transformation with single cloned *R* genes was demonstrated. US 6,127,607 describes resistance proteins with LRR domains and P-loops. The content of US 6,127,607 is herewith incorporated by reference. In particular columns 6 to 8 and col. 11 describe LRR domains and P-loops. Furthermore Song, 2003, PNAS, 100, 16, 9128 shows a comparison of Rpi-blb LRR motifs in Fig. 4 and gives on pages 9132 an overview about LRR domains. The domains of the polypeptid of the present invention are shown in Fig. 14 as well as in Fig. 15.

Preferably the activity of one or more resistance protein(s) selected from the group consisting of Rpi-blb, R1, R-ber, Rpi1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11, Ph-1, Ph-2 and Ph-3 is increased. Preferred is that also the Rpi-blb activity is increased.

In one embodiment of the present invention, the expression of an, e.g. transgenic, Rpi-blb2 protein is increased and further a transgenic resistance gene's expression is increased. The resistance protein coexpressed with the Rpi-blb2 is preferably one of the resistance proteins mentioned herein, in particular Rpi-blb, R1, Rpi1, R-ber, R2, R3, R6, R7, Ph-1, Ph-2 or Ph-3 but can also be one of the others resistance to plant pathogens conferring proteins known to a person skilled in the art.
As mentioned, the term "increased experssion" according to this invention also includes a de novo-Expression of a polynucleotide or polypeptide.

Most preferred is an increase of resistance via coexpression of the polypeptid of the present invention together with Rpi-blb. Rpi-blb and Rpi-blb2 provide both full resistance in detached leaf assays to P. infestans isolates as described in the examples, and in Song 2003, PNAS, 100, 16, 9128.

Said resistance conferring genes are for example described in
RB (synonym of Rpiblb): AY336128 [gi: 32693280], (Song et a., 2003). BAC clones 177 013 and CB3A14 comprising the Rpi-blb gene have been deposited in GenBank with accession nos AY303171 and AY303170.
R1: AF447489 [gi: 9117432422], (Ballvora et a., 2002)
Rpi1: Kuhl, J.C., Hanneman, R.E., and Havey, M.J., (2201) Characterization and mapping pf Rpi1, a late blight resistance locus from diploid (1EBN) Mexican Solanum pinnatisectum. Molecular genet. Genomics 265: 977-985.

R-ber: Ewing, E.E., Simko, I., Smart, C.D., Bonierbale, M.W., Mizubuti, E.S.G., May, G.D., and Fry, W.E., (200) Genetic mapping from field tests of qualitative and quantitative resistance to Phytophthora infestans in a population derived from Solanum tuberosum and Solanum berthaultii. Molecular breeding 6:25-36.

R2: Li, X., vanEck, H.J., vanderVoort, J.N.A.M., Huigen, D.J., Stam, P., and Jacobsen, E. (1998) Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theoretically and Applied Genetics 96 (8): 1121-112.

R3, R6, R7: Elkharbotly, A., Palominosanchez, C., Salamini, F., Jacobsen, E., and Gebhardt, C. (1996) R6 and R7 alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont) de Bary identified genetic loci clustering with the R3 loons on chromosome XI. Theoretical and Applied.Genetics 92 (7): 880-884.

Ph-1: Bonde and Murphy (1952) Main Agric. Exp. Stn. Bull. No 497

Ph-2: Moreau, P., Thoquet, P., Olivier, J., Laterrot, H., and Grimsley, N.H. (1998) Genetic mapping of Ph-2, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. Molecular Plant Microbe Interactions 11 (4): 259-269.

Ph-3: Chunwongse, J., Chunwongse, C., Black, L., and Hanson, P. (2002) Molecular mapping of the Ph-3 gene for late blight resistance in tomato. Journal of Horticultural Science & Biotechnology 77 (3): 281-286.

In one embodiment, the acitivity of the Rpi-blb2 protein is increased or the polynucleotide of the invention is expressed together with a nucleic acid molecule encoding Rpi-blb, R1, R-ber, Rpi1, R2, R3, R6, R7, Ph-1, Ph-2 and/or Ph-3 selected from the group consisting of:

a) nucleic acid molecule encoding at least a mature form of a Rpi-blb (or RB-) polypeptide as encoded by the sequence shown in GenBank Accession no.: AY336128 [gi: 32693280], or the R1 polypeptide as encoded by the sequence shown in GenBank Accession no.: AF447489 [gi 9117432422], or a nucleic acid molecule encoding at least a mature form of a resistance conferring protein mapped and characterized as described for Rpi1 in Kuhl, J.C., Hanneman, R.E., and Havey, M.J., (2001) Characterization and mapping of Rpi1, a late blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Molecular genet. Genomics 265: 977-985; for R-ber in Ewing, E.E., Simko, I., Smart, C.D., Bonierbale, M.W., Mizubuti, E.S.G., May, G.D., and Fry, W.E., (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Molecular breeding 6:25-36; for R2 in Li, X., vanEck, H.J., vanderVoort, J.N.A.M., Huigen, D.J., Stam, P., and Jacobsen, E. (1998) Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theoretically and Applied Genetics 96 (8): 1121-1128, for

R3, R6, R7 in Elkharbotly, A., Palominosanchez, C., Salamini, F., Jacobsen, E., and Gebhardt, C. (1996) R6 and R7 alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont) de Bary identified genetic loci clustering with the R3 locus on chromosome XI. Theoretical and Applied.Genetics 92 (7): 880-884; for Ph-1 in Bonde and Murphy (1952) Main Agric. Exp. Stn. Bull. No 497; for Ph-2 in Moreau, P., Thoquet, P., Olivier, J., Laterrot, H., and Grimsley, N.H. (1998) Genetic mapping of Ph-2, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. Molecular Plant Microbe Interactions 11 (4): 259-269; and for Ph-3 in Chunwongse, J., Chunwongse, C., Black, L., and Hanson, P. (2002) Molecular mapping of the Ph-3 gene for late blight resistance in tomato. Journal of Horticultural Science & Biotechnology 77 (3): 281-286.

b) nucleic acid molecule the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a);

c) nucleic acid molecule encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) or (b) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) or (b);

d) nucleic acid molecule encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a);

e) nucleic acid molecules comprising a fragment or a epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (d);

f) nucleic acid molecule encoding a fragment beginning with amino acid: 1, 30, 50, 100, 200, 500, 1000, or 2000 and stopping with amino acid 2000, 1000, 500, 300, 200, 50, or 1 of a polypeptide encoded by any one of (a) to (e) and with one of said activities;

g) nucleic acid molecule comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (b);

h) nucleic acid molecule encoding a polypeptide being recognized by a monoclonal antibody that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (f);

i) nucleic acid molecule obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (h) or of a fragment thereof of at least 20, preferable 30 or more nucleotides; and

j) nucleic acid molecule the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (i); or the complementary strand of any one of (a) to (j).

Accordingly, the method of present invention confers resistance of one of said plants, plant tissue or plant cell of the present invention to a plant pathogen of a phyllym Oomycetes, preferably to a pathogen of the order Pythiales or Peronosperales, more preferred to the family Pythiaceae or Peronosporaceae, more preferred of the genus Phytophthora or Bremia or Peronospera or Plasmopara, most preferred wherein the pathogen is of the species Phytophthora parasitica var. nicotianae (causing, amongst others, black shank in tobacco), Phytophthora sojae (causing Phytophthora root rot in soybean), Phytophthora capsici (causing rots in pepper and cucurbits and tomato), Phytophthora erythroseptica (causing Pink rot in potato), Plasmopara viticola (causing grapevine downy mildew), Bremia lactuca (causing downy mildew in lettuce) or Peronospora tabaci (causing blue mould in tobacco).

The activity of Rpi-blb2 in a plant, a plant cell, a plant tissue, a plant organ or part thereof according to the present invention can be increased, generated or stimulated via methods which are well known to a person skilled in the art and e.g. are described in Sambrook et al., Cold Spring Harbor Laboratory Press, NY, 1989.

Thus, in a preferred embodiment, the present invention relates to the method of the invention, wherein the expression is a de novo expression.

The term "de novo-Expression" as understood herein relates to a non-detectability of a polypeptide or polynucleotide. Preferred is that no gene encoding a polypeptideor a polynucletide which should be de novo-expressed is present in the genome. However, if the expression can not be detected, it is generally assumed that no expression occurs. A person skilled in the art, however, knows that the detection methods and means develop to higher sensitivity. Thus, de novo-Expression also relates to expression in systems, where the level of expression is to low to confer any resistance to a plant pathogen. A comparison of a knock out strain and a low-expression strain-Phenotyp can show, whether any difference in resistance to any of the herein mentioned pathogens is observable.

Accordingly, in another embodiment of the present invention, the endogenous activity of a Rpi-blb2 and/or a further resistance protein is increased.
The level of expression in a cell can be increased by methods known to a person skilled in tha art. Several techniques are described herein, e.g. the transgenic expresiion of the polynucleotide or polypeptide of the present invention. The polynucleotide or polypeptide can be of foreign origin. Preferred that a polynucleolde of the same genetic origin then the host cell, plant cell, plant tissue, plant is introduced.

The activity, in particular an endogenous activity but also the activity of a transgenic expressed Rpi-blb2 can be increased by several methods. Accordingly, in a preferred embodiment, the activity of the resistance proteins described herein is increased by one or more of the following steps

a) stabilizing the resistance protein;
b) stabilizing the resistance protein encoding mRNA;
c) increasing the specific activity of the resistance protein;
d) expressing or increasing the expression of a homologous or artificial transcription factor for resistance expression;
e) stimulate resistance protein activity through exogenous inducing factors;
f) expressing a transgenic resistance gene; and/or
g) increasing the copy number of the resistance encoding gene.

In general an activity in a organism, in particular in a plant cell, a plant, or a plant tissue can be increased by increasing the amount of the specific protein, i.e. of the resistance protein, in said organism. "Amount of protein" is understood as meaning the amount of a polypeptide, preferably Rpi-blb2, in an organism, a tissue, a cell or a cell compartment. "Increase" of the amount of protein means the quantitative increase of the amount of a protein in an organism, a tissue, a cell or a cell compartment - for example by one of the methods described herein below - in comparison with the wild type of the same genus and species, to which this method had not been applied, under otherwise identical conditions (such as, for example, culture conditions, plant age and the like). The increase amounts to at least 10%, preferably at least 20% or at least 50%, especially preferably at least 70% or 90%, very especially preferably at least 100%, most preferably at least 200% or more.

"Increase" of the activity is understood as meaning the reduction of the total activity of a rotein in an organism, a tissue, a cell or a cell compartment in comparison with the wild type of the same genus and species, to which this method had not been applied, under otherwise identical conditions (such as, for example, culture conditions, plant age and the like). The increase amounts to at least 10%, preferably at least 20% or at least 50%, especially preferably at least 70% or 90%, very especially preferably at least 100%, most preferably at least 200% or more.

In this context, the efficacy of the pathogen resistance can deviate both downward or pward in comparison with a value obtained when increasing one of the Rpi-blb2 proteins as shown in SEQ ID NO: 2 or 4. Preferred functional equivalents are those in which the efficacy of the pathogen resistance measured, for example, by the penetration efficacy of a pathogen or as described herein - differs by not more than 50%, preferably 25%, especially preferably 10% from a comparative value obtained by reducing an Rpi-blb2 protein as shown in SEQ ID NO: 2 or 4. Especially preferred are

ducing an Rpi-blb2 protein as shown in SEQ ID NO: 2 or 4. Especially preferred are those sequences where the increase increases the efficacy of pathogen resistance quantitatively by more than 50%, preferably 100%, especially preferably 500%, very especially preferably 1000% based on a comparative value obtained by reducing one of the Rpi-blb2 proteins as shown in SEQ ID NO: 2 or 4.

Any comparison is preferably carried out under analogous conditions. "Analogous conditions" means that all conditions such as, for example, culture or growing conditions, assay conditions (such as buffer, temperature, substrates, pathogen concentration and the like) are kept identical between the experiments to be compared and that the set-ups differ only by the sequence of the Rpi-blb2 polypeptides to be compared, their organism of origin and, if appropriate, the pathogen. When choosing the pathogen, each comparison requires that the pathogen be chosen which is most similar to the other equivalent, taking into consideration the species specificity.

Due to the increased Rpi-blb2 activity, the resistance of a plant or a part thereof is increased. In a preferred embodiment, the method of the present invention results in reduction in the sporulation index of at least 30% after infection with P. infestans compared to a wild type, more preferred is a reduction of 50%, even more preferred are 70%, even more preferred are more than 80%, more preferred are 85% and 90%. Most preferred is 95% or more.

Accordingly, the present invention also relates to said polynucleotide of the invention, as defined above, is polynucleotide encoding a Rpi-blb2 protein comprising a nucleic acid molecule selected from the group consisting of:

a) nucleic acid molecules encoding at least the mature form of the polypeptide depicted in SEQ ID NO: 2 or 4;
b) nucleic acid molecules comprising the coding sequence as depicted in SEQ ID NO: 1 or 3 or 5 or 6 or encoding at least the mature form of the polypeptide;
c) nucleic acid molecules the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b);
d) nucleic acid molecules encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) to (c) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) to (c);
e) nucleic acid molecules encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a) or (b);

f) nucleic acid molecules comprising a fragment or a epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);

g) nucleic acid molecules comprising a polynucleotide having a sequence of a nucleic acid molecule amplified from a nucleic acid library using the primers as listed in Tab. 3b, preferably ARF1F or ARF1R;

h) nucleic acid molecules encoding polypeptide fragment beginning with amino acid: 1, 30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1276, 1000, 500, 300, 200, 50, or 30 of a polypeptide encoded by any one of (a) to (g);

i) nucleic acid molecules comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (d);

j) nucleic acid molecules encoding a polypeptide being recognized by a monoclonal antibodies that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (h);

k) nucleic acid molecules obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (j) or of a fragment thereof of at least 15, preferable 30, 60, 90 or more nucleotides; and

l) nucleic acid molecules the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);

or the complementary strand of any one of (a) to (l);
or encoding a polypeptide encoded by a segment of chromosome 6 or of linkage group 6 of Solanum bulbocastanum which co-segregates with a marker selected from table 3a or 3b and which mediates resistance to plant pathogens, preferably of the phylum Oomyceta;
and whereby the polynucleotide has not the sequence of Mi1.1 or Mi1.2, as e.g. despicted in Seq. ID NO.: 7 and 9.

In an further embodiment, the polynucleotide of the present invention is derived or isolated from the genome of a organsim selected from the group consisting of Menyanthaceae, Solanaceae, Sclerophylacaceae, Duckeodendraceae, Goetzeaceae, Convolvulaceae, Cuscutaceae, Polemoniaceae, and Hydrophyllaceae according to the Systeme Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof, more preferably it is selected from the group consisting of Atropa, Browallia, Brunfelsia, Capsicum, Cestrum, Cyphomandra, Datura, Fabiana, Franciscea, Hyoscyamus, Lycium, Mandragora, Nicandra, Nicotiana, Petunia, Physalis, Schizanthus and Solanum according to the Systema Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof, even more preferred is a selection out of the group consisting of Solanceae family, preferably S. bulbocastanum, potato (S. tuberosum), tomato (S. lyccopersicum), petunia, tree

tomato (S. betaceum), pear melon (S. muricatum) and eggplant (S. melongena). Even more preferred are tomato or potato or S. bulbocastanum as source for the polynucleotide of the present invention. Most preferred is S. bulbocastanum as source.

Rpi-blb2 has been isolated from S. tuberosum material derived form ABPT. Thus, taxonomic perspective the Rpi-blb2 described is also S. tuberosum-derived. However, the gene was present on an introgression fragment presumably derived from S. bulbocastanum. A lot of S. tuberosom varieties contain introgression fragments of related Solanum species, but still are S. tuberosum. Therefore, S. tuberosum can according to the taxonomical system also be a source for the polynucleotid of the present invention, in particular ABPT-drived S. tuberosum, as well as other varieties of other Solanum species varieties derived in a similar way.
Accordingly, in another embodiment the polynucleotid of the present invention is derived from S. tuberosum.

A polynucleotide of the present invention, e.g., a nucleic acid molecule having a nucleotide sequence of Seq ID NO: 1 or 3 or 5 or 6, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, Rpi-blb2 cDNA can be isolated from a library using all or portion of one of the sequences of the polynucleotide of the present invention as a hybridization probe and standard hybridization techniques (e.g., as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Moreover, a polynucleotide encompassing all or a portion of one of the sequences of the polynucleotide of the present invention can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence (e.g., a nucleic acid molecule encompassing all or a portion of one of the sequences of polynucleotide of the present invention can be isolated by the polymerase chain reaction using oligonucleotide primers as mentioned above, designed based upon this same sequence of polynucleotide of the present invention. For example, mRNA can be isolated from cells, e.g. S. bulbocastanum or another plant (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al. (1979) *Biochemistry* 18: 5294-5299) and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon one of the nucleotide sequences shown in SEQ ID No: 1 or 3 or 5 or 6. A polynucleotide of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplifi-

cation techniques. The polynucleotide so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to an Rpi-blb2 nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In an embodiment of the present invention is the Rpi-blb 2 protein is encoded by a segment of chromosome 6 or linkage group 6 of Solanum bulbocastanum or S. tuberosum.

Further the present invention comprises a segment of chromosome 6 or linkage group 6 of S. bulbocastanum or S. tuberosum. In one preferred embodiment in the method of the present invention the Rpi-blb2 protein expressed is encoded by a polynucleotide comprising a segment of chromosome 6 or linkage group 6 of S. bulbocastanum. Preferably said segment a group comprises further cis acting element, e.g. promoters, enhancers, binding sites etc. or trans acting elements, like cofactors, activators or other resistance proteins, which confer a increased resistance. Genomic fragments comprising the Rpi-blb2 gene and further regulatory elements are depicted in Seq. ID NO.: 5 and 6.

A person skilled in the art knows how to obtain a chromosome segment, e.g. by cloning chromosome fragments into BACs, s. for example Song, 2003, PNAS, 100, 16, 9128 or as described herein and in the references cited herein.

Accordingly, in a further embodiment, the polynucleotide of the present invention or a polynucleotide encoding the Rpi-blb2 protein co-segregates with a marker selected from table 3a or comprises a replication site or hybridization site for said marker. As described in detail in the examples, the resistance to P. infestans could be mapped with the markers depicted in table 3a or 3b. As closer a marker is localized to a gene, as higher is the percentage of lines in which the marker co-sogregates with said marker. Therefore in a preferred embodiment, the polynucleotide of the present invention co-sogregates with the Marker E40M58, CT119 and/or CT216.

In a further embodiment, the present invention relates to a method for making a recombinant vector comprising inserting the polynucleotide of the present invention into a vector or inserting said polynucleotide and a further resistance protein into a vector.

Accordingly, in one further embodiment, the present invention relates to a vector containing the polynucleotide of the present invention or said polynucleotide and a further resistance gene or produced by the method of the present invention.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting a polynucleotide to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA seg-

ments can be ligated. Another type of vector is a viral vector, wherein additional DNA or PNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The present invention also relates to cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering that contain a nucleic acid molecule according to the invention. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, the nucleic acid molecules and vectors of the invention can be reconstituted into liposomes for delivery to target cells.

In another embodiment, the vector of the present invention or the method of the present invention the vector or the method is characterized therein, that the polynucleotide encoding Rpi-blb2 protein or a further resistance protein is operatively linked to expression control sequences and/or a linked to a nucleic acid sequence encoding a transgenic expression regulating signal allowing expression in prokaryotic or eukaryotic host cells.

In a preferred embodiment, the present invention relates to a vector of the present invention or the method of the present invention in which the polynucleotide encoding Rpi-blb2 protein and/or the further resistance protein is operatively linked to expression control sequences of the same species origin as the polynucleotide encoding Rpi-blb2 protein and/or the further resistance protein.

In the case that a nucleic acid molecule according to the invention is expressed in a cell it is in principle possible to modify the coding sequence in such a way that the protein is located in any desired compartment of the plant cell. These include the nucleus,

endoplasmatic reticulum, the vacuole, the mitochondria, the plastids like amyloplasts, chloroplasts, chromoplasts, the apoplast, the cytoplasm, extracellular space, oil bodies, peroxisomes and other compartments of plant cells (for review see Kermode, Crit. Rev. Plant Sci. 15, 4 (1996), 285-423 and references cited therin). The polynucleotide can then operatively be fused to an appropriate polynucleotide, e.g., a vector, encoding a signal for the transport into the desirable compartment.

In an other preferred embodiment of the present invention relates to a vector in which the polynucleotide of the present invention is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic host cells. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes, generally control sequences include promoters, terminators and, in some instances, enhancers, transactivators; or transcription factors.

The term "control sequence" is intended to include, at a minimum, components the presence of which are necessary for expression, and may also include additional advantageous components.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is used.
Operable linkage is to be understood as meaning, for example, the sequential arrangement of a promoter with the nucleic acid sequence to be expressed and, if appropriate, further regulatory elements such as, for example, a terminator in such a way that each of the regulatory elements can fulfil its function when the nucleic acid sequence is expressed recombinantly, depending on the arrangement of the nucleic acid sequences in relation to sense or antisense RNA. To this end, direct linkage in the chemical sense is not necessarily required. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence from positions which are further away, or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 200 base pairs, especially preferably less than 100 base pairs, very especially preferably less than 50 base pairs.

Operable linkage, and an expression cassette, can be generated by means of customary recombination and cloning techniques as are described, for example, in Maniatis T, Fritsch EF and Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML and Enquist LW (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Ausubel FM et al. (1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience and in Gelvin et al. (1990) in: Plant Molecular Biology Manual. However, further sequences which, for example, act as a linker with specific cleavage sites for restriction enzymes, or as a signal peptide, may also be positioned between the two sequences. The insertion of sequences may also lead to the expression of fusion proteins. Preferably, the expression cassette, consisting of a linkage of promoter and nucleic acid sequence to be expressed, can exist in a vector-integrated form and be inserted into a plant genome, for example by transformation.

Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press,Boca Raton, Florida, eds.:Glick and Thompson, Chapter 7, 89-108 including the references therein. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by polynucleotides as described herein.

The recombinant expression vectors of the invention can be designed for expression of said resistance proteins, preferably Rpi-blb2, in prokaryotic or eukaryotic cells. For example, genes encoding the polynucleotide of the invention can be expressed in bacterial cells such as E. coli, *C. glutamicum*, Agrobacterium tumefaciens, insect cells (using baculovirus expression vectors), yeast and other fungal cells (see Romanos, (1992), *Yeast* 8: 423-488; van den Hondel, (1991) J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel, (1991) in: Applied Molecular Genetics of Fungi, Peberdy, eds., p. 1-28, Cambridge University Press: Cambridge), algae (Falciatore et al., 1999, Marine Biotechnology.1, 3:239-251), and multicellular plant cells (see Schmidt, R. (1988), *Plant Cell Rep.*: 583-586); Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, S.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic

Plants, Vol. 1, Engineering and Utilization, eds.:Kung und R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225 (and references cited therein) or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein but also to the C-terminus or fused within suitable regions in the proteins. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Further, the fusion vector can also encode for additional proteins, which expression supports an increase of the activity of Rpi-blb2 or of the resistance of a plant against plant pathogens, e.g. other resistance proteins. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.
Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ).
Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89.

One strategy to maximize recombinant protein expression is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as E. coli or *C. glutamicum* (Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

Further, the vector can be a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA).

Preferably, the polynucleotide of the present invention or described herein is operatively linked to a plant expression control sequences, e.g. expression cassettes plant expression cassette preferably contains regulatory sequences capable to drive gene expression in plants cells and which are operably linked so that each sequence can fulfil its function such as termination of transcription such as polyadenylation signals. Preferred polyadenylation signals are those originating from Agrobacterium tumefaciens t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984), 835 ff) or functional equivalents therof but also all other terminators functionally active in plants are suitable.
As plant gene expression is very often not limited on transcriptional levels as plant expression cassette preferably contains other operably linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranlated leader sequence from tobacco mosaic virus enhancing the protein per RNA ratio (Gallie et al 1987, Nucl. Acids Research 15:8693-8711).

Accordingly, the polynucleotide described herein can be operably linked to an appropriate promoter conferring gene expression in a timely, cell or tissue specific manner. Preferrred are promoters driving constitutitive expression (Benfey et al., EMBO J. 8 (1989) 2195-2202) like those derived from plant viruses like the 35S CAMV (Franck et al., Cell 21(1980) 285-294), the 19S CaMV (see also US5352605 and WO8402913) or plant promoters like those from Rubisco small subunit described in US 4962028.

The term plant-specific promoters is understood as meaning, in principle, any promoter which is capable of governing the expression of genes, in particular foreign genes, in plants or plant parts, plant cells, plant tissues or plant cultures. In this context, expression can be, for example, constitutive, inducible or development-dependent.

The following are preferred:

a) Constitutive promoters

Preferred vectors are those which make possible constitutive expression in plants (Benfey et al.(1989) EMBO J 8:2195-2202). "Constitutive" promoter is understood as meaning those promoters which ensure expression in a large number of, preferably all, tissues over a substantial period of plant development, preferably at all stages of plant development. In particular a plant promoter or a promoter derived from a plant virus are preferably used. Particularly preferred is the promoter of the CaMV cauliflower mosaic virus 35S transcript (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221- 228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202). Another suitable constitutive promoter is the "Rubisco small subunit (SSU)" promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No. X03677), the Agrobacterium nopaline synthase promoter, the TR dual promoter, the Agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker.

b) Tissue-specific promoters

Preferred are furthermore promoters with specificity for the anthers, ovaries, flowers, leaves, stems, roots and seeds.

Seed-specific promoters
such as, for example, the phaseolin promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), the 2S albumin gene promoter (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), the legumin promoter (Shirsat A et al. (1989) Mol Gen Genet 215(2): 326-331), the USP (unknown seed protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), the napin gene promoter (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the sucrose binding protein promoter (WO 00/26388) or the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Baeumlein et al: (1992) Plant Journal 2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980). Further suitable seed-

specific promoters are those of the genes encoding the high-molecular-weight glutenin (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase) or starch synthase. Furthermore preferred are promoters which permit seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. The following can be employed advantageously: the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promoters described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the glutelin gene, the zein gene, the kasirin gene or the secalin gene).

Tuber-, storage-root- or root-specific promoters such as, for example, the patatin promoter class I (B33), the potato cathepsin D inhibitor promoter.

Leaf-specific promoters
such as the potato cytosolic FBPase promoter (WO 97/05900), the Rubisco (ribulose-1,5-bisphosphate carboxylase) SSU (small subunit) promoter or the ST-LSI promoter from potato (Stockhaus et al. (1989) EMBO J 8:2445-2451). Very especially preferred are epidermis-specific promoters such as, for example, the OXLP gene (oxalate-oxidase-like protein) promoter (Wei et al. (1998) Plant Mol. Biol. 36:101-112).

Flower-specific promoters
such as, for example, the phytoene synthase promoter (WO 92/16635) or the promoter of the P-rr gene (WO 98/22593).

Anther-specific promoters
such as the 5126 promoter (US 5,689,049, US 5,689,051), the glob-1 promoter and the γ-zein promoter.

c) Chemically inducible promoters

The expression cassettes can also comprise a chemically inducible promoter (review article: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108), by which the expression of the exogenous gene in the plant at a particular point in time can be controlled. Such promoters such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), a salicylic-acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic-acid-inducible promoter (EP 0 335 528) or an ethanol- or cyclohexanone-inducible promoter (WO 93/21334) can likewise be used.

d) Stress- or pathogen-inducible promoters

Further preferred promoters are those which are induced by biotic or abiotic stress such as, for example, the pathogen-inducible promoter of the PRP1 gene (Ward et al. (1993) Plant Mol Biol 22:361-366), the tomato high-temperature-inducible hsp70 or hsp80 promoter (US 5,187,267), the potato low-temperature-inducible alpha-amylase promoter (WO 96/12814), the light-inducible PPDK promoter, or the wounding-induced pinII promoter (EP375091).

Pathogen-inducible promoters encompass those of genes which are induced as a consequence of infection by pathogens, such as, for example, genes of PR proteins, SAR proteins, β-1,3-glucanase, chitinase and the like (for example Redolfi et al. (1983) Neth J Plant Pathol 89:245-254; Uknes, et al. (1992) The Plant Cell 4:645-656; Van Loon (1985) Plant Mol Viral 4:111-116; Marineau et al. (1987) Plant Mol Biol 9:335-342; Matton et al. (1987) Molecular Plant-Microbe Interactions 2:325-342; Somssich et al. (1986) Proc Natl Acad Sci USA 83:2427-2430; Somssich et al. (1988) Mol Gen Genetics 2:93-98; Chen et al. (1996) Plant J 10:955-966; Zhang and Sing (1994) Proc Natl Acad Sci USA 91:2507-2511; Warner, et al. (1993) Plant J 3:191-201; Siebertz et al. (1989) Plant Cell 1:961-968(1989).

Also encompassed are wounding-inducible promoters such as that of the pinII gene (Ryan (1990) Ann Rev Phytopath 28:425-449; Duan et al. (1996) Nat Biotech 14:494-498), of the wun1 and wun2 gene (US 5,428,148), of the win1 and win2 gene (Stanford et al. (1989) Mol Gen Genet 215:200-208), of systemin (McGurl et al. (1992) Science 225:1570-1573), of the WIP1 gene (Rohmeier et al. (1993) Plant Mol Biol 22:783-792; Eckelkamp et al. (1993) FEBS Letters 323:73-76), of the MPI gene (Corderok et al. (1994) The Plant J 6(2):141-150) and the like.

e) Development-dependent promoters

Further suitable promoters are, for example, fruit-maturation-specific promoters such as, for example, the tomato fruit-maturation-specific promoter (WO 94/21794, EP 409 625). Development-dependent promoters comprise partly the tissue-specific promoters, since individual tissues develop by nature in a development-dependent fashion.

It can be advantageously that the polypeptide of the present invention is only active or has only an increased activity in the tissue which is transfected or penetrated by the pathogen mentioned herein. Especially preferred are constitutive promoters and leaf- and/or stem-specific, pathogen-inducible and epidermis-specific promoters, with pathogen-inducible and epidermis-specific promoters being most preferred. Also preferred is

the natural promoter, which is e.g. comprised in the genomic fragment depicted in Seq. ID NO.: 5 and 6.

Furthermore, further promoters may be linked operably to the nucleic acid sequence to be expressed, which promoters make possible the expression in further plant tissues or in other organisms, such as, for example, E. coli bacteria. Suitable plant promoters are, in principle, all of the above-described promoters.

The term "genetic control sequences" is to be understood in the broad sense and refers to also all those sequences which have an effect on the materialization or the function of the expression cassette according to the invention. For example, genetic control sequences modify the transcription and translation in prokaryotic or eukaryotic organisms. Preferably, the expression cassettes according to the invention encompass the promoter with specificity for the embryonal epidermis and/or the flower 5'-upstream of the nucleic acid sequence in question to be expressed recombinantly, and 3'-downstream a terminator sequence as additional genetic control sequence and, if appropriate, further customary regulatory elements, in each case linked operably to the nucleic acid sequence to be expressed recombinantly.
Genetic control sequences also encompass further promoters, promoter elements or minimal promoters, all of which can modify the expression-governing properties. Thus, for example, the tissue-specific expression may additionally depend on certain stressors, owing to genetic control sequences. Such elements have been described, for example, for water stress, abscisic acid (Lam E and Chua NH, J Biol Chem 1991; 266(26): 17131 -17135) and heat stress (Schoffl F et al., Molecular & General Genetics 217(2-3):246-53, 1989).

Further advantageous control sequences are, for example, the Gram-positive promoters amy and SPO2, and the yeast or fungal promoters ADC1, MFa , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH.

In principle, all natural promoters with their regulatory sequences like those mentioned above may be used for the method according to the invention. In addition, synthetic promoters may also be used advantageously.

Genetic control sequences furthermore also encompass the 5'-untranslated regions, introns or noncoding 3'-region of genes, such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (general reference: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). It has been demonstrated that they may play a significant role in the regulation of gene expression. Thus, it has been demonstrated that 5'-untranslated sequences can enhance the transient expression of

heterologous genes. Examples of translation enhancers which may be mentioned are the tobacco mosaic virus 5' leader sequence (Gallie et al. (1987) Nucl Acids Res 15:8693-8711) and the like. Furthermore, they may promote tissue specificity (Rouster J et al. (1998) Plant J 15:435-440).

The expression cassette may advantageously comprise one or more of what are known as enhancer sequences, linked operably to the promoter, which make possible an increased recombinant expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminators, may also be inserted at the 3' end of the nucleic acid sequences to be expressed recombinantly. One or more copies of the nucleic acid sequences to be expressed recombinantly may be present in the gene construct.

Polyadenylation signals which are suitable as control sequences are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular gene 3' of the T-DNA (octopin synthase) of the Ti plasmid pTiACHS (Gielen et al. (1984) EMBO J 3:835 et seq.) or functional equivalents thereof. Examples of terminator sequences which are especially suitable are the OCS (octopin synthase) terminator and the NOS (nopalin synthase) terminator.

Control sequences are furthermore to be understood as those which make possible homologous recombination or insertion into the genome of a host organism or which permit removal from the genome. In the case of homologous recombination, for example the natural promoter of a particular gene may be exchanged for a promoter with specificity for the embryonal epidermis and/or the flower. Methods such as the cre/lox technology permit a tissue-specific, if appropriate inducible, removal of the expression cassette from the genome of the host organism (Sauer B (1998) Methods. 14(4):381-92). In this method, specific flanking sequences (lox sequences), which later allow removal by means of cre recombinase, are attached to the target gene.

An expression cassette and the vectors derived from it may comprise further functional elements. The term functional element is to be understood in the broad sense and refers to all those elements which have an effect on the generation, amplification or function of the expression cassettes, vectors or transgenic organisms according to the invention. The following may be mentioned by way of example, but not by limitation:

a) Selection markers which confer a resistance to a metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456), antibiotics or biocides, preferably herbicides, such as, for example, kanamycin, G 418, bleomycin or hygromycin,

or else phosphinothricin and the like. Especially preferred selection markers are those which confer resistance to herbicides. Examples which may be mentioned are: DNA sequences which encode phosphinothricin acetyl transferases (PAT) and which inactivate glutamin synthase inhibitors (bar and pat genes), 5-enol-pyruvylshikimate-3-phosphate synthase genes (EPSP synthase genes), which confer resistance to Glyphosater (N-(phosphonomethyl)glycine), the gox gene, which encodes Glyphosater-degrading enzymes (Glyphosate oxidoreductase), the deh gene (encoding a dehalogenase which inactivates dalapon), sulfonyl-urea- and imidazolinone-inactivating acetolactate synthases, and bxn genes, which encode bromoxynil-degrading nitrilase enzymes, the aasa gene, which confers resistance to the antibiotic apectinomycin, the streptomycin phospho-transferase (SPT) gene, which allows resistance to streptomycin, the neomycin phosphotransferase (NPTII) gene, which confers resistance to kanamycin or geneticidin, the hygromycin phosphotransferase (HPT) gene, which mediates resistance to hygromycin, the acetolactate synthase gene (ALS), which confers resistance to sulfonylurea herbicides (for example mutated ALS variants with, for example, the S4 and/or Hra mutation).

b) Reporter genes which encode readily quantifiable proteins and, via their color or enzyme activity, make possible an assessment of the transformation efficacy, the site of expression or the time of expression. Very especially preferred in this context are genes encoding reporter proteins (Schenborn E, Groskreutz D. Mol Biotechnol. 1999; 13(1):29-44) such as the green fluorescent protein (GFP) (Sheen et al.(1995) Plant Journal 8(5):777-784; Haseloff et al.(1997) Proc Natl Acad Sci USA 94(6):2122-2127; Reichel et al.(1996) Proc Natl Acad Sci USA 93(12):5888-5893; Tian et al. (1997) Plant Cell Rep 16:267-271; WO 97/41228; Chui WL et al. (1996) Curr Biol 6:325-330; Leffel SM et al. (1997) Biotechniques. 23(5):912-8), chloramphenicol transferase, a luciferase (Ow et al. (1986) Science 234:856-859; Millar et al. (1992) Plant Mol Biol Rep 10:324-414), the aequorin gene (Prasher et al. (1985) Biochem Biophys Res Commun 126(3):1259-1268), β-galactosidase, R locus gene (encoding a protein which regulates the production of anthocyanin pigments (red coloring) in plant tissue and thus makes possible the direct analysis of the promoter activity without addition of further auxiliary substances or chromogenic substrates; Dellaporta et al., In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium, 11:263-282, 1988), with β-glucuronidase being very especially preferred (Jefferson et al., EMBO J. 1987, 6, 3901-3907).

c) Origins of replication, which ensure amplification of the expression cassettes or vectors according to the invention in, for example, E. coli. Examples which may

be mentioned are ORI (origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

d) Elements which are necessary for Agrobacterium-mediated plant transformation, such as, for example, the right or left border of the T-DNA or the vir region.

To select cells which have successfully undergone homologous recombination, or else to select transformed cells, it is, as a rule, necessary additionally to introduce a selectable marker, which confers resistance to a biocide (for example herbicide), a metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456) or an antibiotic to the cells which have successfully undergone recombination. The selection marker permits the selection of the transformed cells from untransformed ones (McCormick et al. (1986) Plant Cell Reports 5:81-84).

The introduction of an expression cassette according to the invention into an organism or cells, tissues, organs, parts or seeds thereof (preferably into plants or plant cells, tissue, organs, parts or seeds) can be effected advantageously using vectors which comprise the expression cassettes. The expression cassette can be introduced into the vector (for example a plasmid) via a suitable restriction cleavage site. The plasmid formed is first introduced into E. coli. Correctly transformed E. coli are selected, grown, and the recombinant plasmid is obtained by the methods familiar to the skilled worker. Restriction analysis and sequencing may serve to verify the cloning step.

Further promoters for expression in specific plant parts are e.g. the napin-gene promoter from rapeseed (US5608152), the USP-promoter from Vicia faba (Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67), the oleosin-promoter from Arabidopsis (WO9845461), the phaseolin-promoter from Phaseolus vulgaris (US5504200), the Bce4-promoter from Brassica (WO9113980) or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9) as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (WO9515389 and WO9523230) or those desribed in WO9916890 (promoters from the barley hordein-gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the Sorghum kasirin-gene, the rye secalin gene).

Further, the polynucleotide of the invention can be cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA mole-

cule) of an RNA molecule which is antisense to the mRNA encoded by the polynucleotide of the present invention. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acid molecules are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986 and Mol et al., 1990, FEBS Letters 268:427-430.

In one embodiment the present invention relates to a method of making a recombinant host cell comprising introducing the vector or the polynucleotide of the present invention or said vector or said polynucleotide and a vector for expressing a further resistance protein into a host cell.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection", conjugation and transduction are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemical-mediated transfer, or electroporation. Suitable methods for transforming or transfecting host cells including plant cells can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, ed: Gartland and Davey, Humana Press, Totowa, New Jersey.

For stable transfection of enkaryontic cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate or in plants that confer resistance towards a herbicide such as glyphosate or glufosinate. Nucleic acid encoding a selectable marker can be introduced

into a host cell on the same vector as that encoding the polypeptide of the present invention or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by, for example, drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

Further host cells can be produced which contain selection systems which allow for regulated expression of the introduced gene. For example, inclusion of the polynucleotide of the invention on a vector placing it under control of the lac operon permits expression of the polynucleotide only in the presence of IPTG. Such regulatory systems are well known in the art.

Preferably, the introduced nucleic acid molecule is foreign to the host cell.

By "foreign" it is meant that the nucleic acid molecule is either heterologous with, respect to the host cell, this means derived from a cell or organism with a different genomic background, or is homologous with respect to the host cell but located in a different genomic environment than the naturally occurring counterpart of said nucleic acid molecule. This means that, if the nucleic acid molecule is homologous with respect to the host cell, it is not located in its natural location in the genome of said host cell, in particular it is surrounded by different genes. In this case the nucleic acid molecule may be either under the control of its own promoter or under the control of a heterologous promoter. The vector or nucleic acid molecule according to the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained in some form extrachromosomally. In this respect, it is also to be understood that the nucleic acid molecule of the invention can be used to restore or create a mutant gene via homologous recombination (Paszkowski (ed.), Homologous Recombination and Gene Silencing in Plants. Kluwer Academic Publishers (1994)).

Accordingly, in another embodiment the present invention relates to a host cell genetically engineered with the polynucleotide of the invention or the vector of the invention, or said vector or said polynucleotide and a vector or a polynucleotide for expressing a further resistance protein.

The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

For example, an polynucleotide of the present invention can be introduced in bacterial cells, insect cells, fungal cells or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells), algae, ciliates, plant cells or fungi. Suitable host cells are known to those skilled in the art. Preferred are E. coli, baculovirus, Agrobacterium or plant cells.

Further, the host cell can also be transformed such that further enzymes and proteins are (over)expressed which expression supports an increase of resistance of a plant to pathogens. Preferably, a further resistance genes is also expressed, preferably one or more genes are as mentioned herein is/are expressed. Most preferred is a coexpression of Rpi-blb2 and Rpi-blb.

Further preferred are cells of one of herein mentioned plants, in particular, of one of the above-mentioned Solanaceae, most preferred are potato, tomato, petunia, tree tomato, pear melon or egg plant.

In another embodiment, the present invention relates to a process for the production of the polypeptide of the present invention, in particular of a protein having Rpi-blb2 activity comprising culturing the host cell of the invention and recovering the polypeptide encoded by said polynucleotide and expressed by the host cell from the culture or the cells.

The term "expression" means the production of a protein or nucleotide sequence in the cell. However, said term also includes expression of the protein in a cell-free system. It includes transcription into an RNA product, post-transcriptional modification and/or translation to a protein product or polypeptide from a DNA encoding that product, as well as possible post-translational modifications.

Depending on the specific constructs and conditions used, the protein may be recovered from the cells, from the culture medium or from both. For the person skilled in the art it is well known that it is not only possible to express a native protein but also to express the protein as fusion polypeptides or to add signal sequences directing the protein to specific compartments of the host cell, e.g., ensuring secretion of the protein into the culture medium; etc. Furthermore, such a protein and fragments thereof can be chemically synthesized and/or modified according to standard methods described, for example hereinbelow.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) the polypeptide encoded by the polynucleotide of the invention, preferably a polypeptid having Rpi-blb2 activity. An alternate method can be

applied in addition in plants by the direct transfer of DNA into developing flowers via electroporation or Agrobacterium medium gene transfer. Accordingly, the invention further provides methods for producing Rpi-blb2 using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention in a suitable medium such that the polypeptid of the present invention is produced. Further, the method comprises isolating recovering said polypeptid from the medium or the host cell.

The polypeptide of the present invention is preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector (as described above), the expression vector is introduced into a host cell (as described above) and said polypeptide is expressed in the host cell. Said polypeptide can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques. Alternative to recombinant expression, the polypeptide or peptide of the present invention can be synthesized chemically using standard peptide synthesis techniques. Moreover, native Rpi-blb2 can be isolated from cells (e.g., endothelial cells), for example using the antibody of the present invention as described below, in particular, an anti-Rpi-blb2 antibody, which can be produced by standard techniques utilizing the polypeptid of the present invention or fragment thereof, i.e., the polypeptide of this invention.

In one embodiment, the present invention relates to a Rpi-blb2 protein or a protein having Rpi-blb2 activity.

In one embodiment, the present invention relates to a polypeptide having the amino acid sequence encoded by a polynucleotide of the invention or obtainable by a process of the invention.

The terms "protein" and "polypeptide" used in this application are interchangeable. "Polypeptide" refers to a polymer of amino acids (amino acid sequence) and does not refer to a specific length of the molecule. Thus peptides and oligopeptides are included within the definition of polypeptide. This term does also refer to or include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

Preferably, the polypeptide is isolated. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material when produced by re-

combinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

The language "substantially free of cellular material" includes preparations of the polypeptide of the invention in which the protein is separated from cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations having less than about 30% (by dry weight) of "contaminating protein", more preferably less than about 20% of "contaminating protein", still more preferably less than about 10% of "contaminating protein", and most preferably less than about 5% "contaminating protein". The term "Contaminating protein" relates to polypeptides which are not polypeptides of the present invention. When the polypeptide of the present invention or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations in which the polypeptide or of the present invention is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. The language "substantially free of chemical precursors or other chemicals" includes preparations having less than about 30% (by dry weight) of chemical precursors or non-Rpi-blb2 chemicals, more preferably less than about 20% chemical precursors or non- Rpi-blb2 chemicals, still more preferably less than about 10% chemical precursors or non- Rpi-blb2 chemicals, and most preferably less than about 5% chemical precursors or non- Rpi-blb2 chemicals. In preferred embodiments, isolated proteins or biologically active portions thereof lack contaminating proteins from the same organism from which the polypeptide of the present invention is derived. Typically, such proteins are produced by recombinant

A polypeptide of the invention can participate in the polypeptide or portion thereof comprises preferably an amino acid sequence which is sufficiently homologous to an amino acid sequence of SEQ ID No: 2 or 4 such that the protein or portion thereof maintains the ability to confer the resistance of the present invention. The portion of the protein is preferably a biologically active portion as described herein. Preferably, the polypeptide of the invention has an amino acid sequence identical as shown in SEQ ID No: 2 or 4. Further, the polypeptide can have an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, preferably hybridizes under stringent conditions as described above, to a nucleotide sequence of the polynucleotide of the present invention. Accordingly, the polypeptide has an amino acid sequence which is encoded by a nucleotide sequence that is at least about 70%, preferably at least about 75%, more preferably at least about 80%, 90%, 95%, and even more preferably at least about

96%, 97%, 98%, 99% or more homologous to one of the amino acid sequences of SEQ ID No: 2 or 4. The preferred polypeptide of the present invention preferably possess at least one of the Rpi-blb2 activities described herein, e.g. its resistance or immunological acitivities. A preferred polypeptide of the present invention includes an amino acid sequence encoded by a nucleotide sequence which hybridizes, preferably hybridizes under stringent conditions, to a nucleotide sequence of SEQ ID No: 1 or 3 or 5 or 6 or which is homologous thereto, as defined above.

Accordingly the polypeptide of the present invention can vary from SEQ ID No: 2, or 4 in amino acid sequence due to natural variation or mutagenesis, as described in detail herein. Accordingly, the polypeptide comprise an amino acid sequence which is at least about 70%, preferably at least about 75%, and more preferably at least about 80, 90, 95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of SEQ ID No:1 or 3 or 5 or 6.

Biologically active portions of an polypeptide of the present invention include peptides comprising amino acid sequences derived from the amino acid sequence of an Rpi-blb2, e.g., the amino acid sequence shown in SEQ ID No: 2 or 4 or the amino acid sequence of a protein homologous thereto, which include fewer amino acids than a full length Rpi-blb2 or the full length protein which is homologous to an Rpi-blb2 depicted herein, and exhibit at least one activity of Rpi-blb2. Typically, biologically (or immunologically) active portions i.e. peptides, e.g., peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length comprise a domain or motif with at least one activity or epitope of an Rpi-blb2. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein.

Manipulation of the Rpi-blb2 polynucleotide of the invention may result in the production of Rpi-blb2 having functional differences from the wild-type Rpi-blb2. These proteins may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

Any mutagenesis strategies for Rpi-blb2 to result in increased said resistance or a resistance to another plant pathogen species or an other strain of a plant pathogen species aforementioned, of said compound are not meant to be limiting; variations on these strategies will be readily apparent to one skilled in the art. Using such strategies, and incorporating the mechanisms disclosed herein, the polynucleotide and polypeptide of the invention may be utilized to generate plants or parts thereof, expressing wildtyp Rpi-blb2 or mutated Rpi-blb2 polynucleotide and protein molecules such that

the yield, production, and/or efficiency of production of a desired compound is improved. This desired compound may be any natural product of plants, which includes the final products of biosynthesis pathways and intermediates of naturally-occurring metabolic pathways, as well as molecules which do not naturally occur in the metabolism of said cells, but which are produced by a said cells of the invention.

The invention also provides chimeric or fusion proteins.

As used herein, an "chimeric protein" or "fusion protein" comprises an polypeptide operatively linked to a non- Rpi-blb2 polypeptide.

An "Rpi-blb2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to polypeptide having a Rpi-blb2, whereas a "non-Rpi-blb2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the Rpi-blb2, e.g., a protein which does not confer the resistance described herein, in particular does not confer resistance to P. infestans and which is derived from the same or a different organism.
Within the fusion protein, the term "operatively linked" is intended to indicate that the Rpi-blb2 polypeptide and the non-Rpi-blb2 polypeptide are fused to each other so that both sequences fulfil the proposed function addicted to the sequence used. The non-Rpi-blb2 polypeptide can be fused to the N-terminus or C-terminus of the Rpi-blb2 polypeptide. For example, in one embodiment the fusion protein is a GST-LMRP fusion protein in which the Rpi-blb2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant Rpi-blb2. In another embodiment, the fusion protein is an Rpi-blb2 containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of an Rpi-blb2 can be increased through use of a heterologous signal sequence.
Preferably, an Rpi-blb2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. The fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992).

Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). The polynucleotide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the encoded protein.

Furthermore, folding simulations and computer redesign of structural motifs of the protein of the invention can be performed using appropriate computer programs (Olszewski, Proteins 25 (1996), 286-299; Hoffman, Comput. Appl. Biosci. 11 (1995), 675-679). Computer modeling of protein folding can be used for the conformational and energetic analysis of detailed peptide and protein models (Monge, J. Mol. Biol. 247 (1995), 995-1012; Renouf, Adv. Exp. Med. Biol. 376 (1995), 37-45). In particular, the appropriate programs can be used for the identification of interactive sites of mitogenic cyplin and its receptor, its ligand or other interacting proteins by computer assistant searches for complementary peptide sequences (Fassina, Immunomethods (1994), 114-120. Further appropriate computer systems for the design of protein and peptides are described in the prior art, for example in Berry, Biochem. Soc. Trans. 22 (1994), 1033-1036; Wodak, Ann. N. Y. Acad. Sci. 501 (1987), 1-13; Pabo, Biochemistry 25 (1986), 5987-5991. The results obtained from the above-described computer analysis can be used for, e.g., the preparation of peptidomimetics of the protein of the invention or fragments thereof. Such pseudopeptide analogues of the, natural amino acid sequence of the protein may very efficiently mimic the parent protein (Benkirane, J. Biol. Chem. 271 (1996), 33218-33224). For example, incorporation of easily available achiral Q-amino acid residues into a protein of the invention or a fragment thereof results in the substitution of amide bonds by polymethylene units of an aliphatic chain, thereby providing a convenient strategy for constructing a peptidomimetic (Banerjee, Biopolymers 39 (1996), 769-777).

Superactive peptidomimetic analogues of small peptide hormones in other systems are described in the prior art (Zhang, Biochem. Biophys. Res. Commun. 224 (1996), 327-331). Appropriate peptidomimetics of the protein of the present invention can also be identified by the synthesis of peptidomimetic combinatorial libraries through successive amide alkylation and testing the resulting compounds, e.g., for their binding and immunological properties. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, Methods in Enzymology 267 (1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715.

Furthermore, a three-dimensional and/or crystallographic structure of the protein of the invention can be used for the design of peptidomimetic inhibitors of the biological activity of the protein of the invention (Rose, Biochemistry 35 (1996), 12933-12944; Rutenber, Bioorg. Med. Chem. 4 (1996),1545-1558).

In a further embodiment, the present invention relates to an antibody that binds specifically to the polypeptide of the present invention or parts, i.e. specific fragments or epitopes of such a protein.

The antibodies of the invention can be used to identify and isolate Rpi-blb2 and genes in any organism, preferably plants, prepared in plants described herein. These antibodies can be monoclonal antibodies, polyclonal antibodies or synthetic antibodies as well as fragments of antibodies, such as Fab, Fv or scFv fragments etc. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfr6, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals.

Furthermore, antibodies or fragments thereof to the aforementioned peptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. These antibodies can be used, for example, for the immunoprecipitation and immunolocalization of proteins according to the invention as well as for the monitoring of the synthesis of such proteins, for example, in recombinant organisms, and for the identification of compounds interacting with the protein according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies selections, yielding a high increment of affinity from a single library of phage antibodies which bind to an epitope of the protein of the invention (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13). In many cases, the binding phenomena of antibodies to antigens is equivalent to other ligand/anti-ligand binding.

In one embodiment, the present invention relates to an antisense nucleic acid molecule comprising the complementary sequence of the polypeptide of the present invention. Methods to modify the expression levels and/or the activity are known to persons skilled in the art and include for instance overexpression, co-suppression, the use of ribozymes, sense and anti-sense strategies, gene silencing approaches. "Sense strand" refers to the strand of a double-stranded DNA molecule that is homologous to a mRNA transcript thereof. The "anti-sense strand" contains an inverted sequence which is complementary to that of the "sense strand".

An "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a "sense" nucleic acid molecule encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid molecule can hydrogen bond to a sense nucleic acid molecule. The antisense nucleic acid molecule can be complementary to an entire Rpi-blb2 coding strand, or to only a portion thereof. Accordingly, an antisense nucleic acid molecule can be antisense to a "coding region" of the coding

strand of a nucleotide sequence of a polynucleotide of the present invention. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. Further, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding Rpi-blb2. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into a polypeptide, i.e., also referred to as 5' and 3' untranslated regions (5'-UTR or 3'-UTR).

Given the coding strand sequences encoding Rpi-blb2 disclosed herein, antisense nucleic acid molecules of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of Rpi-blb2 mRNA, but can also be an oligonucleotide which is antisense to only a portion of the coding or noncoding region of Rpi-blb2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of Rpi-blb2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid molecule of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid molecule (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a polynucleotide has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleotide will be of an antisense orientation to a target polynucleotide of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an Rpi-blb2 to thereby inhibit expression of the protein. e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic including plant promoters are preferred.

In a further embodiment, the antisense nucleic acid molecule of the invention can be an -anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

Further the antisense nucleic acid molecule of the invention can be a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave Rpi-blb2 mRNA transcripts to thereby inhibit translation of mRNA. A ribozyme having specificity for an Rpi-blb2 -encoding nucleic acid molecule can be designed based upon the nucleotide sequence of an Rpi-blb2 cDNA disclosed herein or on the basis of a heterologous sequence to be isolated according to methods taught in this invention. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an encoding mRNA..See, e.g., Cech et al. U.S. Patent No. 4,987,071 and Cech et al. U.S. Patent No. 5,116,742. Alternatively, Rpi-blb2 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

The antisense molecule of the present invention comprises also a polynucleotide comprising a nucleotide sequences complementary to the regulatory region of an Rpi-blb2

nucleotide sequence, e.g., its promoter and/or enhancers, e.g. to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In addition, in one embodiment, the present invention relates to a method for the production of transgenic plants, plant cells or plant tissue comprising the introduction of the polynucleotide or the vector of the present invention into the genome of said plant, plant tissue or plant cell. In a preferred embodiment, said vector or said polynucleotide and a vector or a polynucleotide for the expression of a further resistance gene, in particular for Rpi-blb, is also introduced into the genome of said plant, plant tissue or plant cell, before, after or together.

For the expression of the nucleic acid molecules according to the invention in sense or antisense orientation in plant cells, the molecules are placed under the control of regulatory elements which ensure the expression in plant cells. These regulatory elements may be heterologous or homologous with respect to the nucleic acid molecule to be expressed as well with respect to the plant species to be transformed and are described above in detail.

In general, such regulatory elements comprise a promoter active in plant cells. To obtain expression in all tissues of a transgenic plant, e.g. constitutive promoters are used, such as the 35 S promoter of CaMV (Odell, Nature 313 (1985), 810-812) or promoters of the polyubiquitin genes of maize (Christensen, Plant Mol. Biol. 18 (1982), 675-689). In order to achieve expression in specific tissues of a transgenic plant it is possible to use tissue specific promoters (see, e.g., Stockhaus, EMBO J. 8 (1989), 2245-2251). Known are also promoters which are specifically active in tubers of potatoes or in seeds of different plants species, such as maize, Vicia, wheat, barley etc. Inducible promoters may be used in order to be able to exactly control expression. Inducible promoters comprise also promoters, which are induced by infections of plants. Further embodiments are described above.

In one embodiment, the present invention relates to a method for producing a plant or a part thereof resistant to a pathogen of the phylum Oomyceta comprising the steps: expressing in the plant or a part thereof the polypeptide of the present invention and a further resistance protein.

Accordingly in one further embodiment, the present invention relates to transgenic plant or plant tissue of the invention or produced according to the method of the invention, which upon the presence of the polynucleotide or the vector is resistant to said pathogens.

The generation of a transformed organism (or of a transformed cell or tissue) requires introducing the DNA, RNA or protein in question into the relevant host cell.
A multiplicity of methods are available for this procedure, which is termed transformation (or transduction or transfection) (Keown et al. (1990) Methods in Enzymology 185:527-537). For example, the DNA or RNA can be introduced directly by microinjection or by bombardment with DNA-coated microparticles. Also, the cell can be permeabilized chemically, for example using polyethylene glycol, so that DNA can enter the cell by diffusion. The DNA can also be introduced by protoplast fusion with other DNA-containing units such as minicells, cells, lysosomes or liposomes. Another suitable method of introducing DNA is electroporation, where the cells are permeabilized reversibly by an electrical pulse. Suitable methods have been described (for example by Bilang et al. (1991) Gene 100:247-250; Scheid et al. (1991) Mol Gen Genet 228:104-112; Guerche et al. (1987) Plant Science 52:111-116; Neuhause et al. (1987) Theor Appl Genet 75:30-36; Klein et al. (1987) Nature 327:70-73; Howell et al. (1980) Science 208:1265; Horsch et al.(1985) Science 227:1229-1231; DeBlock et al. (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

In plants, the above-described methods of transforming and regenerating plants from plant tissues or plant cells are exploited for transient or stable transformation. Suitable methods are especially protoplast transformation by polyethylene-glycol-induced DNA uptake, the biolistic method with the gene gun, what is known as the particle bombardment method, electroporation, incubation of dry embryos in DNA-containing solution, and microinjection.
In addition to these "direct" transformation techniques, transformation can also be effected by bacterial infection by means of Agrobacterium tumefaciens or Agrobacterium rhizogenes. The Agrobacterium-mediated transformation is best suited to dicotyledonous plant cells. The methods are described, for example, by Horsch RB et al. (1985) Science 225: 1229f.
When agrobacteria are used, the expression cassette must be integrated into specific plasmids, either into a shuttle or intermediate vector, or into a binary vector. If a Ti or Ri plasmid is to be used for the transformation, at least the right border, but in most cases the right and left border, of the Ti or Ri plasmid T-DNA is linked to the expression cassette to be introduced in the form of a flanking region.

Binary vectors are preferably used. Binary vectors are capable of replication both in E. coli and in Agrobacterium. As a rule, they comprise a selection marker gene and a linker or polylinker flanked by the right and left T-DNA border sequence. They can be transferred directly into Agrobacterium (Holsters et al. (1978) Mol Gen Genet 163:181-

187). The selection marker gene permits the selection of transformed agrobacteria and is, for example, the nptII gene, which confers resistance to kanamycin. The Agrobacterium which acts as host organism in this case should already contain a plasmid with the vir region. The latter is required for transferring the T-DNA to the plant cell. An Agrobacterium transformed in this way can be used for transforming plant cells. The use of T-DNA for transforming plant cells has been studied and described intensively (EP 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasserdam, Chapter V; An et al. (1985) EMBO J 4:277-287). Various binary vectors are known, some of which are commercially available such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA).

Further promoters which are suitable for expression in plants have been described (Rogers et al. (1987) Meth in Enzymol 153:253-277; Schardl et al. (1987) Gene 61:1-11; Berger et al. (1989) Proc Natl Acad Sci USA 86:8402-8406).

Direct transformation techniques are suitable for any organism and cell type.

The plasmid used need not meet any particular requirements in the case of the injection or electroporation of DNA or RNA into plant cells. Simple plasmids such as those of the pUC series can be used. If complete plants are to be regenerated from the transformed cells, it is necessary for an additional selectable marker gene to be located on the plasmid.

Stably transformed cells, i.e. those which contain the introduced DNA integrated into the DNA of the host cell, can be selected from untransformed cells when a selectable marker is part of the DNA introduced. Examples of genes which can act as markers are all those which are capable of conferring resistance to antibiotics or herbicides (such as kanamycin, G 418, bleomycin, hygromycin or phosphinothricin) (see above). Transformed cells which express such marker genes are capable of surviving in the presence of concentrations of a corresponding antibiotic or herbicide which kill an untransformed wild type. Examples are mentioned above and preferably comprise the bar gene, which confers resistance to the herbicide phosphinothricin (Rathore KS et al. (1993) Plant Mol Biol 21(5):871-884), the nptII gene, which confers resistance to kanamycin, the hpt gene, which confers resistance to hygromycin, or the EPSP gene, which confers resistance to the herbicide Glyphosate. The selection marker permits the selection of transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5:81-84). The resulting plants can be bred and hybridized in the customary fashion. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

The abovementioned methods are described, for example, in Jenes B et al.(1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization. edited by SD Kung and R Wu, Academic Press, pp. 128-143 and in Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225). The construct to be expressed is preferably cloned into a vector which is suitable for the transformation of Agrobacterium tumefaciens, for example pBin19 (Bevan et al. (1984) Nucl Acids Res 12:8711f).

As soon as a transformed plant cell has been generated, a complete plant can be obtained using methods known to the skilled worker. For example, callus cultures are used as starting material. The development of shoot and root can be induced in this as yet undifferentiated cell biomass in a known fashion. The shoots obtained can be planted out and bred.

The skilled worker is familiar with such methods of regenerating intact plants from plant cells and plant parts. Methods to do so are described, for example, by Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al (1995) Plant Cell Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533.

The method according to the invention can advantageously be combined with further methods which bring about pathogen resistance (for example to insects, fungi, bacteria, nematodes and the like), stress resistance or another improvement of the plant properties. Examples are mentioned, inter alia, by Dunwell JM, Transgenic approaches to crop improvement, J Exp Bot. 2000;51 Spec No; pages 487-96.

Suitable strains of Agrobacterium tumefaciens and vectors as well as transformation of Agrobacteria and appropriate growth and selection media are well known to those skilled in the art and are described in the prior art (GV31 01 (pMK90RK), Koncz, Mol. Gen. Genet. 204 (1986), 383-396; C58C1 (pGV 3850kan), Deblaere, Nucl. Acid Res. 13 (1985), 4777; Bevan, Nucleic. Acid Res. 12(1984), 8711; Koncz, Proc. Natl. Acad. Sci. USA 86 (1989), 8467-8471; Koncz, Plant Mol. Biol. 20 (1992), 963-976; Koncz, Specialized vectors for gene tagging and expression studies. In: Plant Molecular Biology Manual Vol 2, Gelvin and Schilperoort (Eds.), Dordrecht, The Netherlands: Kluwer Academic Publ. (1994), 1-22; EP-A-120 516; Hoekema: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasserdam (1985), Chapter V, Fraley, Crit. Rev. Plant. Sci., 4, 1-46; An, EMBO J. 4 (1985), 277-287).
Although the use of Agrobacterium tumefaciens is preferred in the method of the invention, other Agrobacterium strains, such as Agrobacterium rhizogenes, may be used, for example if a phenotype conferred by said strain is desired.

The transformation of most dicotyledonous plants is possible with the methods described above. But also for the transformation of monocotyledonous plants several successful transformation techniques have been developed. These include the transformation using biolistic methods as, e.g., described above as well as protoplast transformation, electroporation of partially permeabilized cells, introduction of DNA using glass fibers, etc.
The term "transformation" as used herein, refers to the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for the transfer. The polynucleotide may be transiently or stably introduced into the host cell and may be maintained non-integrated, for example, as a plasmid or as chimeric links, or alternatively, may be integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known by a skilled person.

Accordingly, in one embodiment, the present invention relates to a plant cell comprising the polynucleotide the vector of the present invention or obtainable by the method of the present invention. Preferably, the cell comprises a further resistance conferring polynucleotide or vector, more preferred is a Rpi-blb encoding vector or polynucleotide.

Thus, the present invention relates also to transgenic plant cells which contain (preferably stably integrated into the genome) a polynucleotide according to the invention linked to regulatory elements which allow expression of the polynucleotide in plant cells and wherein the polynucleotide is foreign to the transgenic plant cell. For the meaning of foreign; see supra.
Thus, the present invention also relates to transgenic plants and plant tissue comprising transgenic plant cells according to the invention. Due to the (over)expression of a polypeptide of the invention, said plant or plant tissues are resistance to plant pathogens, in particular to Oomyceta. Preferably the plants are also resistance to other pathogen, e.g. to sucking plant pathogens. Further pathogens are described herein. Preferred is that said plants or plant tissue is resistance to Phythophthora species, most preferred to P. infestans.
For example, to obtain transgenic plants expressing the Rpi-blb2 gene, its coding-region can be cloned, e.g., into the pBinAR vector (Höfgen und Willmitzer, Plant-Science, 66, 1990, 221-230). For example, following a polymerase chain reaction (PCR) technology the coding region of Rpi-blb2 can be amplified using Primers as shown in the examples and figures, e.g., in Table 3a in particular ARF1F and ARF1R. The obtained PCR fragment can be purified and subsequently the fragment can be cloned into a vector. The resulted vector can be transferred into Agrobacterium turnefaciens. This strain can be used to transform and transgenic plants can then be selected in another embodiment, the present invention relates to a transgenic plant or plant tissue comprising the plant cell of the present invention.

"Transgenic", for example regarding a nucleic acid sequence, an expression cassette or a vector comprising said nucleic acid sequence or an organism transformed with said nucleic acid sequence, expression cassette or vector, refers to all those constructs originating by recombinant methods in which either

a) the Rpi-blb2 nucleic acid sequence, or

b) a genetic control sequence linked operably to the RacB nucleic acid sequence, for example a promoter, or

c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, an example of a modification being a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment refers to the natural chromosomal locus in the organism of origin, or to the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least at one side and has a sequence of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp, in length. A naturally occurring expression cassette - for example the naturally occurring combination of the Rpi-blb2 promoter with the corresponding Rpi-blb2 gene - becomes a transgenic expression cassette when it is modified by non-natural, synthetic "artificial" methods such as, for example, mutagenization. Such methods have been described (US 5,565,350; WO 00/15815; also see above).

Further, the plant cell, plant tissue or plant can also be transformed such that further enzymes and proteins are (over)expressed which expression supports an increase of the plant's or the plant tissue's resistance, for example Rpi-blb, R1, R-ber, Rpi1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11, Ph-1, Ph-2 and/or Ph-3-proteins. Preferred is the coexpression of Rpi-blb and Rpi-blb2.

The present invention also relates to cultured plant tissues comprising transgenic plant cells as described above which show expression of a protein according to the invention.

Host or starting organisms which are preferred as transgenic organisms are mainly plants in accordance with the above definition. Included within the scope of the invention are all genera and species of higher and lower plants of the Plant Kingdom. Furthermore included are the mature plants, seed, shoots and seedlings, and parts, propagation material and cultures derived therefrom, for example cell cultures. Mature

plants refers to plants at any developmental stage beyond that of the seedling. The term seedling refers to a young immature plant in an early developmental stage.

Any transformed plant obtained according to the invention can be used in a conventional breeding scheme or in in vitro plant propagation to produce more transformed plants with the same characteristics and/or can be used to introduce the same characteristic in other varieties of the same or related species. Such plants are also part of the invention. Seeds obtained from the transformed plants genetically also contain the same characteristic and are part of the invention. As mentioned before, the present invention is in principle applicable to any plant and crop that can be transformed with any of the transformation method known to those skilled in the art.

In general, the plants which can be modified according to the invention and which either show overexpression of a protein according to the invention or a reduction of the synthesis of such a protein can be derived from any desired plant species. They can be monocotyledonous plants or dicotyledonous plants, preferably they belong to plant species of interest in agriculture, wood culture or horticulture interest, such as crop plants (e.g. maize, rice, barley, wheat, rye, oats etc.), potatoes, oil producing plants (e.g. oilseed rape, sunflower, pea nut, soy bean, etc.), cotton, sugar beet, sugar cane, leguminous plants (e.g. beans, peas etc.), wood producing plants, preferably trees, etc. However, plants which can be infected by Phythophtora species are preferred.

Accordingly, in one embodiment the plant, plant cell or plant tissue of the invention or produced according to the method of the invention is selected from the group consisting of Menyanthaceae, Solanaceae, Sclerophylacaceae, Duckeodendraceae, Goetzeaceae, Convolvulaceae, Cuscutaceae, Polemoniaceae, and Hydrophyllaceae according to the Systeme Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof. Preferably said plant, plant cell or plant tissue of the invention or produced according to the method of the invention is a Solanaceae, preferably selected from the group of Atropa, Browallia, Brunfelsia, Capsicum, Cestrum, Cyphomandra, Datura, Fabiana, Franciscea, Hyoscyamus, Lycium, Mandragora, Nicandra, Nicotiana, Petunia, Physalis, Schizanthus and Solanum according to the Systema Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof.
More preferred, the plant, plant cell or plant tissue of the invention or produced according to the method of the present invention is a S. bulbocastanum, S. tuberosum (potato), S. lycopersicum, petunia, S. betaceum (tree tomato), S. muricatum (pear melon) or S.melongena (eggplant). Even more preferred, the plant, plant tissue or plant cell is a S. tuberosum or S. lycopersicum. Most preferred is S. tuberosum. In other systems, the classification will be similar. The person skilled in the art knows the differences, e.g.

more common, tomato is named systematicly Lycopersicon Lycopersicum (L.) Karsten ex Farwell.

In yet another aspect, the invention also relates to harvestable parts and to propagation material of the transgenic plants according to the invention which either contain transgenic plant cells expressing a nucleic acid molecule according to the invention or which contains cells which show a reduced level of the described protein.

Harvestable parts can be in principle any useful parts of a plant, for example, flowers, pollen, seedlings, tubers, leaves, stems, fruit, seeds, roots etc. Propagation material includes, for example, seeds, fruits, cuttings, seedlings, tubers, rootstocks etc. Preferred are potatoes, tomatoes, egg fruits or pear melons as harvestable or propagation material. In case, the plant of the invention is petunia, the present invention relates in one embodiment to the flowers of petunia as harvesteable part.

The invention furthermore relates to the use of the transgenic organisms according to the invention and of the cells, cell cultures, parts - such as, for example, roots, leaves and the like in the case of transgenic plant organisms - derived from them, and to transgenic propagation material such as seeds or fruits, for the production of foodstuffs or feeding stuffs, pharmaceuticals or fine chemicals. In particular, potatoes can serve for the production of fine chemicals.

Accordingly in another embodiment, the present invention relates to the use of the polynucleotide, the plant, plant cell or plant tissue, the vector, or the polypeptide of the present invention for making fatty acids, carotenoids, isoprenoids, vitamins, lipids, wax esters, (poly)saccharides and/or polyhydroxyalkanoates, and/or its metabolism products, in particular, steroid hormones, cholesterol, prostaglandin, triacylglycerols, bile acids and/or ketone bodies producing cells, tissues and/or plants. There are a number of mechanisms by which the yield, production, and/or efficiency of production of fatty acids, carotenoids, isoprenoids, vitamins, wax esters, lipids, (poly)saccharides and/or polyhydroxyalkanoates, and/or its metabolism products, in particular, steroid hormones, cholesterol, triacylglycerols, prostaglandin, bile acids and/or ketone bodies or further of above defined fine chemicals incorporating such an altered protein can be affected. In the case of plants, by e.g. increasing the expression of acetyl-CoA which is the basis for many products, e.g., fatty acids, carotenoids, isoprenoids, vitamines, lipids, (poly)saccharides, wax esters, and/or polyhydroxyalkanoates, and/or its metabolism products, in particular, prostaglandin, steroid hormones, cholesterol, triacylglycerols, bile acids and/or ketone bodies in a cell, it may be possible to increase the amount of the produced said compounds thus permitting greater ease of harvesting and purification or in case of plants more efficient partitioning.Further, one or more of said metabo-

lism products, increased amounts of the cofactors, precursor molecules, and intermediate compounds for the appropriate biosynthetic pathways maybe required. Therefore, by increasing the number and/or activity of transporter proteins involved in the import of nutrients, such as carbon sources (i.e., sugars), nitrogen sources (i.e., amino acids, ammonium salts), phosphate, and sulfur, it may be possible to improve the production of acetyl CoA and its metabolism products as mentioned above, due to the removal of any nutrient supply limitations on the biosynthetic process. In particular, it may be possible to increase the yield, production, and/or efficiency of production of said compounds, e.g. fatty acids, carotenoids, isoprenoids, vitamins, was esters, lipids, (poly)saccharides, and/or polyhydroxyalkanoates, and/or its metabolism products, in particular, steroid hormones, cholesterol, prostaglandin, triacylglycerols, bile acids and/or ketone bodies molecules etc. in plants.

Furthermore preferred is a method for the recombinant production of pharmaceuticals or fine chemicals in host organisms, wherein a host organism is transformed with one of the above-described expression cassettes and this expression cassette comprises one or more structural genes which encode the desired fine chemical or catalyze the biosynthesis of the desired fine chemical, the transformed host organism is cultured, and the desired fine chemical is isolated from the culture medium. This method can be applied widely to fine chemicals such as enzymes, vitamins, amino acids, sugars, fatty acids, and natural and synthetic flavorings, aroma substances and colorants. Especially preferred is the production of tocopherols and tocotrienols and carotenoids. The transformed host organisms are cultured and the products are isolated from the host organisms or the culture medium by methods known to the skilled worker. The production of pharmaceuticals such as, for example, antibodies or vaccines, is described by Hood EE, Jilka JM. Curr Opin Biotechnol. 1999 Aug; 10(4):382-6; Ma JK, Vine ND. Curr Top Microbiol Immunol. 1999; 236:275-92.

In one embodiment, the present invention also relates to the use of the polynucleotide, the vector, or the polypeptide of the present invention for producing a plant or a plant tissue, plant organ, or a plant cell or a part thereof resistant to said.

Furthermore, in one embodiment, the present invention relates to a method for the identification of an compound stimulating resistance to a said plant pathogen comprising:

a) contacting cells which express the polypeptide of the present invention or its mRNA with a candidate compound under cell cultivation conditions;
b) assaying an increase in expression of said polypeptide or said mRNA;

c) comparing the expression level to a standard response made in the absence of said candidate compound; whereby, an increased expression over the standard indicates that the compound is stimulating resistance.

Said compound may be chemically synthesized or microbiologically produced and/or comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms, e.g. pathogens. Furthermore, said compound(s) may be known in the art but hitherto not known to be capable of suppressing or activating Rpi-blb2. The reaction mixture may be a cell free extract or may comprise a cell or tissue culture. Suitable set ups for the method of the invention are known to the person skilled in the art and are, for example, generally described in Alberts et al., Molecular Biology of the Cell, third edition (1994), in particular Chapter 17. The compounds may be, e.g., added to the reaction mixture, culture medium, injected into the cell or sprayed onto the plant.

If a sample containing a compound is identified in the method of the invention, then it is either possible to isolate the compound from the original sample identified as containing the compound capable of activating or increasing resistance to said pathogens, or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. Preferably, the compound identified according to the above described method or its derivative is further formulated in a form suitable for the application in plant breeding or plant cell and tissue culture.

The compounds which can be tested and identified according to a method of the invention may be expression libraries, e.g., cDNA expression libraries, peptides, proteins, nucleic acids, antibodies, small organic compounds, hormones, peptidomimetics, PNAs or the like (Milner, Nature Medicine 1 (1995), 879-880; Hupp, Cell 83 (1995), 237-245; Gibbs, Cell 79 (1994), 193-198 and references cited supra). Said compounds can also be functional derivatives or analogues of known inhibitors or activators. Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used, for example, according to the methods described above. The

cell or tissue that may be employed in the method of the invention preferably is a host cell, plant cell or plant tissue of the invention described in the embodiments hereinbefore.

Determining whether a compound is capable of suppressing or activating said resistance can be done, as described in the examples, in particular via sporulation index determination. The activator identified by the above-described method may prove useful as a fungicide or crop protectants. Thus, in a further embodiment the invention relates to a compound obtained or identified according to the method of the invention said compound being an agonist of Rpi-blb2.

Accordingly, in one embodiment, the present invention further relates to a compound identified by the method of the present invention.

Said compound is, for example, a homologous of Rpi-blb2. Homologues of the polypeptid of the present invention can be generated by mutagenesis, e.g., discrete point mutation or truncation of Rpi-blb2. As used herein, the term "homologue" refers to a variant form of the protein which acts as an agonist of the activity of the Rpi-blb2. An agonist of said protein can retain substantially the same, or a subset, of the biological activities of Rpi-blb2

In one embodiment, the invention relates to an antibody specifically recognizing the compound of the present invention.

The invention also relates to a diagnostic composition comprising at least one of the aforementioned polynucleotides, nucleic acid molecules, vectors, proteins, antibodies or compounds of the invention and optionally suitable means for detection.

The diagnostic composition of the present invention is suitable for the isolation of mRNA from a cell and contacting the mRNA so obtained with a probe comprising a nucleic acid probe as described above under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the protein in the cell. Further methods of detecting the presence of a protein according to the present invention comprises immunotechniques well known in the art, for example enzyme linked immunosorbent assay. Furthermore, it is possible to use the nucleic acid molecules according to the invention in particular the markers described in the examples, e.g. in table 3a or 3b as molecular markers or primer in plant breeding. Suitable means for detection are well known to a person skilled in the arm, e.g. buffers and solutions for hydridization assays, e.g. the aforementioned solutions and buffers, further and means for Southern-, Western-, Northern- etc. –blots, as e.g. described in Sambrook et al. are known.

In another embodiment, the present invention relates to a kit comprising the polynucleotide, the vector, the host cell, the polypeptide, the antisense nucleic acid, the antibody, plant cell, the plant or plant tissue, the harvestable part, the propagation material or the compound of the invention.

The compounds of the kit of the present invention may be packaged in containers such as vials, optionally with/in buffers and/or solution. If appropriate, one or more of said components may be packaged in one and the same container. Additionally or alternatively, one or more of said components may be adsorbed to a solid support as, e.g. a nitrocellulose filter, a glas plate, a chip, or a nylon membrane or to the well of a micro titerplate. The kit can be used for any of the herein described methods and embodiments, e.g. for the production of the host cells, transgenic plants, pharmaceutical compositions, detection of homologous sequences, identification of antagonists or agonists, etc.

Further, the kit can comprise instructions for the use of the kit for any of said embodiments, in particular for its use for increasing the resistance to one or more of said pathogens of a plant cell, plant tissue or plant.

In a preferred embodiment said kit comprises further a polynucleotide encoding one or more of the aforementioned resistance protein, preferably Rpi-blb, and/or an antibody, a vector, a host cell, an antisense nucleic acid, a plant cell or plant tissue or a plant related to said resistance protein(s), preferably to Rpi-blb.

In a further embodiment, the present invention relates a method for the production of a crop protectant providing the polynucleotide, the vector or the polypeptide of the invention or comprising the steps of the method of the invention; and formulating the polynucleotide, the vector or the polypeptide of the invention or the compound identified in step (c) of said method in a form applicable as plant agricultural composition.

In another embodiment, the present invention relates to a method for the production of a crop protectant composition comprising the steps of the method of the present invention; and

(a) formulating the compound identified in step (c) in a form acceptable as agricultural composition.

Under "acceptable as agricultural composition" is understood, that such a composition is in agreement with the laws regulating the content of fungicides, plant nutrients, herbizides, etc. Preferably such a composition is without any harm for the protected plants and the animals (humans included) fed therewith.

The present invention also pertains to several embodiments relating to further uses and methods. The polynucleotide, polypeptide, protein homologues, fusion proteins, primers, vectors, host cells, described herein can be used in one or more of the following methods: identification of plants resistant to plant pathogens as mentioned and related organisms; mapping of genomes; identification and localization of sequences of interest; evolutionary studies; determination of regions required for function; modulation of an activity.

Accordingly, the polynucleotides of the present invention have a variety of uses. First, they may be used to identify an organism as being S. bulbocastanum or a close relative thereof. Also, they may be used to identify the presence of S. bulbocastanum or a relative thereof in a mixed population of microorganisms. By probing the extracted genomic DNA of a culture of a unique or mixed population of plants under stringent conditions with a probe spanning a region of the gene of the present invention which is unique to this S. bulbocastanum, one can ascertain whether the present invention has been used or whether S. bulbocastanum or a close relative is present.

Further, the polynucleotide of the invention may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related organism.
The polynucleotides of the invention are also useful for evolutionary and protein structural studies. By comparing the sequences of the Rpi-blb2 of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the protein which are essential for the functioning of the enzyme. This type of determination is of value for protein engineering studies and may give an indication of what the protein can tolerate in terms of mutagenesis without losing function.

These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on the Internet, for example under http://www.ncbi.nlm.nih.gov/PubMed/medline.html. Further databases and addresses, such as http://www.ncbi.nlm.nih.gov/, http://www.infobiogen.fr/, http://www.fmi.ch/biology/research-tools.html, http://www.tigr.org/, are known to the person skilled in the art and can also be obtained using, e.g., http://www.lycos.com. An overview of patent information in biotechnology and a survey of relevant sources of

patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

Tables:

Table 1: Sequences:

Table 2. Segregation of resistance in 2851 progeny clones of BC4 mapping populations ARG 95-3 and ARP 96-11 in the field trial of 2000 at Marknesse, The Netherlands. Numbers of clones classified as having a resistant, susceptible of unknown phenotype is presented with percentages in parenthesis.

Mapping population	No clones with susceptible phenotype	No clones with resistant phenotype	No clones with unknown phenotype	Totals
ARG 95-3	846 (37)	886 (39)	551 (24)	2283
ARP 96-11	256 (45)	170 (30)	142 (25)	568
Totals	1102 (39)	1056 (37)	693 (24)	2851

Table 3A. Overview of markers used for mapping *Rpiblb2*

Marker	Ori[1]	Sequence	Annealing temp (°C)	Restriction Enzyme[2]
E46M52	F	TTGTGGTTATCGATGAGAAT	56,5	SCAR (b)
	R	GAAACAACAGCAGGATAGTGAG		
E46M52e	F	TTGTGGTTATCGATGAGAAT	61	SCAR (a,b);MboI (c)
	R	GAAACAACAGCAGGATAGTGAG		
E40M58	F	GAATTCAGCACAAATACCAA	50	DdeI (a)
	R	TTAACGTTTACTATCACGAG		
E40M58e	F	GTAGAAACAGCAGCCTCATAAGC	55	SCAR (a)
	R	TTCTGCCTAATTGCCCTGTG		
S1E00	F	GGGGTTGGGAAGACAACGACAC	50	AFLP
	R	AATTCCAAGATACAGTCAAATAC		
41L	F	AGGCAGGATTAACAGTAGAAG	58	TaqI (a)
	R	CATGCTTTTAGGAAGAAGCTC		
38L	F	TTGAGACAAAGCAGCTCCAC	59	ApoI (a,b)
	R	ACGTTTCTCACACCTACAGG		
69L	F	TGATGGCACGTTTGATCGTG	61	TaqI (a,b);HpaII (c)
	R	TAAGATCCAAACCAGCCACC		
69R	F	CCTTATCACACATGTGGCTAC	58	RsaI(a,b); ApoI (c)
	R	ATTGAAACGGAGGAAGTACAAC		
141R	F	TTCTTCATATGGCAGACCAAC	60	RsaI (a,b); DdeI (c)
	R	CTACTCTGCTGACATGCAGG		

24L	F	GAGATTCTCAAAGGTGTCTTCC	60	SCAR (a,b,c)
	R	AACCTGTGCTTTCCCATTCG		
24R	F	CTTTCACAAGCGTCACTTTGG	58	SCAR (a,b)
	R	TAAAAAGAATCAACAGGGCAAC		
14L	F	ACGACTGCTCAAAGTTGGCC	58	SCAR (a,b,c)
	R	CCAAGAAGCCAGTTGAGAGC		
123L	F	GTAGATTACACTATGGATATGG	60	SCAR (a,b)
	R	CAGTTAGCAGCAATGTCAGC		
123L2	F	CATTCAACTAGGCCAAAAGTGG	59	SCAR (a,b); DraI (c)
	R	CCAGGTAGGTGTTTTCTTCC		
123R	F	GTTCTAAGTCAGATGCCACC	62	SCAR (a,b)
	R	AAGTGCTCCAACACGAGCC		
133R	F	TGAGTTCTCTTACCCTGCG	60	SCAR (a,b)
	R	GGATATCCAGCATCAATGCC		
133R2	F	GGTGAGCCTCCTTGCATTCC	60	SCAR (a,b)
	R	CCTGAGGGAAGATGTCACG		
99L	F	CCTAGTTTAGAGTGAGTAGAC	58	SCAR (a,b)
	R	GTGATATATTGCTCAAGGATCC		
113R	F	GTTGCTGGCTGTCACTGATC	59	SCAR (a,b)
	R	GTGATGTGCAGGGTTCAAGG		
67L	F	GATTAGTGTAGATCTTAGCTTG	62	MboI (a,b)
	R	AAATCTCTCTCACAATTATCCC		
112L	F	CTATTGACTGAACCTGCTGAG	56	HaeIII (a); HinfI (c)
	R	TGAAGTCATTTAGTCCACAGC		
CT216 (RFLP)	F	AGATCGGAGTGTGAACATGG	56	
	R	CTTCTACTTCTAGTCGACTGC		
CT216	F	CGTAGTCCATCTGAAGCTCC	65	SCAR (a,b)
	R	TCTTCTTCTGCTAGTCGTCG		
CT119	F	ACTATTCTCACGTAAGGGGACAC	60	HindIII (a,b)
	R	GTGTACATGTATGAAACTCTAGC		
CT119N	F	GTTCCTTTCAATCAGAAAGTAG (aro 120)	55	SCAR (a)
	R	CTTTGGATGAGTCAAAAGGCT (aro 121)		
14L24L	F	univ14L	60	CfoI (c)
	R	univ24L		
SPB30L	F	CAAGTTACGGCAACCAAGAG	57	HpaII (c)
	R	CTTTGACACAGTGTTAGAATGC		
SPB39L	F	CGTGATCTAGGAGTTACGAC	52	SCAR (c)
	R	CTTATTTTAAATACAAGACATCTGG		
24L9spec	F	univ. 14L	56	HhaI (c)
	R	CAGAGGAAAGTCAACCAACG		
24Lspec	F	univ. 14L	60	CfoI (c)
	R	CAGAGGAAAGTCAACCAACG		
NptII	F	TCGGCTATGACTGGGCACAACAGA	70	
	R	AAGAAGGCGATAGAAGGCGATGCG		

M13	F	TGTAAAACGACGGCCAGT	55
	R	GGAAACAGCTATGACCATG	

[1] Ori: Orientation of the primer; F: forward primer; R: reverse primers
[2] a: ARG95-3, b; ARP96-11, c: B6a

Tabel 3B. Overview of primers used for mapping *Rpi-blb2*

primer	Ori	Sequence[1]
ARO 73	F	TTCAGCACAAATACCAAT
ARO 74	R	GATGTTCCCCTTCTTTTA
ARO 77	R	TTGTGGTTATCGATGAGAAT
ARO 79	R	ACCTGGCGTTCCTTATTTTT
ARO 94		NGTCASWGANAWGAA
ARO 128	F	GATGGAGCGGAAAAGCCGGTG
ARO 129	F	GGTGTTTTGTAGCATCTCCAG
ARO 295		CCATGATTACGCCAAGCTGG
ARO 296		GGTTTTCCCAGTCACGACGT
univ14L	F	AGAAAGCTCACCAGTGGACC
univ24L	R	ATTTATGGCTGCAGAGGACC
123Mi	R	AAGTCCAATTGCTCATCCATC
14L2	R	TGCACCATGCACGAAGGTC
24L2	F	CAATWTTGGTTCCCGAAATTGG
ARF1F	F	ATGGAAAAACGAAAAGATAATGAAG
ARF1R	R	CTACTTAAATAACGGGATATCCTTC
ARO 602	F	CCCATGACTCCTTGAGTTTG
S1		GGTGGGGTTGGGAAGACAACG
*Eco*R1+0		GTAGACTGCGTACCAATTC
*Mse*I+0		GATGAGTCCTGAGTAA

[1] N=A+T+G+C, S=G+C,W=A+T

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Table 4. Complementation of late blight susceptibility in potato

			cv Impala		cv Kondor	
BAC-library	Source BAC	Genotype[1]	RGC-containing plants/ transformants	R plants/ RGC-containing plants	RGC-containing plants/ transformants	R plants/ RGC-containing plants
ARD 1197-16	24	R_0 (*RGC1*)	12/15[a]	0/12		
			8/10[b]	0/8		
	24	R_0 (*RGC2*)	8/11[a]	0/8		
			5/6[b]	0/5		
	24	R_0 (*RGC3*)	11/13[a]	0/11		
			5/7[b]	0/5		
	211	R_0 (*RGC4*)	5/7[a]	0/5	10/12[a]	0/10
	242	R_0 (*RGC4*)	5/7[a]	0/5	8/8[a]	0/8
	211	R_0 (*RGC5*)	5/7[a]	4/5	12/13[a]	12/12
	211	R_0 (*RGC6*)	-	-	-	-
	211	R_0 (*RGC24L*)	-	-	-	
Blb 2002	SPB39	R_0 (*RGC4*)	5/6[a]	0/5	3/3[a]	0/3
	SPB39	R_0 (*RGC5*)	11/15[a]	11/11	8/8[a]	7/8
	SPB39	R_0 (*RGC6*)	3/3[a]	0/3	6/6[a]	0/6
	SPB30	R_0 (*RGC7*)	3/4[a]	0/3	9/9[a]	0/9
	SPB30	R_0 (*RGC8*)	-	-	-	-
	SPB39	R_0 (24L)	-		-	
		R_0 (pBINPLUS)	3/3	0/3	8/10	0/8

1 R_0 genotypes are primary transformants obtained from transformation of the susceptible potato cultivars Impala or Kondor with T-DNA constructs containing the *Rpi-blb2* gene candidates *RGC1* to *RGC8* and *RGC24L* or an empty pBINPLUS vector. *Agrobacterium tumefaciens* strains UIA143[a] or AGL0[b] were used for transformation of the *P. infestans* susceptible potato cultivars Impala and Kondor.

Table 5. Cycling conditions used for TAIL-PCR

Reaction	cycle no.	Thermal condition
Primary	1	92°C (2 min), 95°C (1 min)
	5	94°C (15s), 63°C (1 min), 72°C (2 min)
	1	94°C (15s), 30°C (3 min), ramping to 72°C over 3 min, 72°C (2 min)
	10	94°C (5s), 44°C (1 min),72°C (2 min)
	12[a]	94°C (5s), 63°C (1 min), 72°C (2 min)
		94°C (5s), 63°C (1 min), 72°C (2 min)
		94°C (5s), 44°C (1 min), 72°C (2 min)
	1	72°C (5 min)
Secondary	10[a]	94°C (5s), 63°C (1 min), 72°C (2 min)
		94°C (5s), 63°C (1 min), 72°C (2 min)
		94°C (5s), 44°C (1 min), 72°C (2 min)
	1	72°C (5 min)
Tertiary	20	94°C (10s), 44°C (1 min), 72°C (2 min)
	1	72°C (5 min)

[a] these are nine-segment super cycles each consisting of two high-stringency and one reduced-stringency cycle

The figures show:

Figure 1. Schematic representation of the development of the complex interspecific hybrid clones designated as 'ABPT' (1a) and the *S. tuberosum* mapping populations that were derived from two of these clones: ABPT clone 55 and ABPT clone 60 (1b to d). A; *Solanum acaule*, B; *S. bulbocastanum*, P; *S. pureja*, T; *S. tubersosum*, 2x : diploid (2n=2x=24), 3x; triploid, 4x ; tetraploid, 6x ; hexaploid, cv; cultivar. Codes in italics indicate mapping populations.

Figure 2. Disease progress curves for clone ARF 87-601 and susceptible control cultivars (cv) Bildtstar, Eersteling and the partial resistant control cultivar Pimpernel in a field test for foliar resistance to late blight in Toluca Valley, Mexico in 1991. At eight time points after planting, the percentage blighted foliage due to a natural late blight infection was scored on the 1 to 9 CIP scale (Estrada-Ramos, 1983).

Figure 3. Disease progress curves for clone ARF 87-507, ARF 87-601, ARF 87-801, the susceptible control cultivar (cv) Granola and the partial resistant breeding clone AR 85-96-13 in a field test for foliar resistance to late blight in Benguet Province, Philippines in 1992. At six time points between August 25th to November 24th, the percentage blighted foliage due to a natural late blight infection was scored on the 1 to 9 CIP scale (Estrada-Ramos, 1983).

Figure 4. Typical phenotypes in tetraploid resistant and susceptible parental clones and progeny clones segregating for *Rpi-blb2* mediated resistance to late blight in the annual field trial at Marknesse, The Netherlands, approximately 6 weeks after inoculation with isolate IPO82001 of *P. infestans*. Six plant plots with a clone showing the resistant phenotype (within black solid line) that shows no or hardly any sporulating lesions and with a clone showing the susceptible phenotype (within white dotted line) that shows completely blighted foliage.

Figure 5. Genetic map based on 109 progeny clones of *S. tuberosum* mapping population *ARG 95-15* showing 7 AFLP markers that were found to cosegregate with the *Rpi-blb2* locus. Numbers left to the vertical line indicate the genetic distance between flanking markers or the *Rpi-blb2* locus in centimorgan (cM).

Figure 6. Genetic map based on 137 progeny clones of *S. tuberosum* mapping population *ARG 95-3* showing 15 AFLP markers and RGA marker S1E00 that were found to cosegregate with the *Rpi-blb2* locus. Phenotypes of the progeny clones were obtained with detached leaf assays. Numbers left to the vertical line indicate the genetic distance between flanking markers or the *Rpi-blb2* locus in centimorgan (cM).

Figure 7. Genetic map based on 178 progeny clones of *S. tuberosum* mapping population *ARG 95-3* showing 5 markers that were found to cosegregate with the *Rpi-blb2* locus on linkage group 6 of *S. tuberosum*. Phenotypes of the progeny clones were determined in the field trial at Marknesse, the Netherlands in 1998. Markers E40M58 and E46M52 were scored either as AFLP, CAPS, SCAR or extended (suffix: e) marker (table 3A). Partly, marker CT119 was scored as marker CT119N (table 3a). Marker CT216 was scored as SCAR marker. The number left to the vertical line indicates the genetic distance between flanking markers or the *Rpi-blb2* locus in centimorgan (cM). For each marker, the number of recombinants between marker and phenotype and the total number of progeny clones scored is given in parenthesis.

Figure 8. Genetic maps based on 886 progeny clones of *S. tuberosum* mapping population *ARG 95-3* and on 170 progeny clones of *S. tuberosum* mapping population *ARP 96-11*, showing markers that were found to cosegregate with the *Rpi-blb2* locus

on linkage group 6 of *S. tuberosum*. Phenotypes of the progeny clones were determined in the field trial at Marknesse, the Netherlands in 2000. The number left to the vertical line indicates the genetic distance between flanking markers in centimorgan (cM). The marker interval which delimitates the position of the *Rpi-blb2* gene, based on detected recombination events in progeny clones, is indicated by double arrow headed lines.

Figure 9. Physical map of the genomic region containing *Rpi-blb2* in *S. tuberosum* (upper horizontal line) and *S. bulbocastanum* (lower horizontal line). Vertical lines indicate the relative position of markers linked to resistance. Numbers above the horizontal lines are the number of recombinants identified between the flanking markers in 1056 and 1899 progeny plants of *S. tuberosum*, derived from complex species hybrids "ABPT" (Figure 1), and *S. bulbocastanum* progeny plants respectively. ABPT-derived progeny comprises clones from both the mapping populations *ARG 95-3* and *ARP 96-11*. Rectangles represent bacterial artificial chromosome (BAC) clones from the ARD 1197-16 BAC library except for BAC clones with prefix "Blb" which were from the *S. bulbocastanum* Blb 2002 BAC library. The marker interval which delimitates the position of the *Rpi-blb2* gene, based on detected recombination events in progeny clones, is indicated by double arrow headed lines. Small arrows indicate positions of Resistance Gene Candidates (RGC's).

Figure 10. Schematic representation of the development of the diploid, intraspecifc mapping population *B6* of *S. bulbocastanum*. Codes in italics indicate mapping populations.

Figure 11. Genetic map based on 1899 progeny clones of *S. bulbocastanum* mapping population *B6*, showing markers that were found to cosegregate with the *Rpi-blb2* locus on chromosome 6 of *S. bulbocastanum*. Phenotypes of the progeny clones were determined by detached leaf assays. The number left to the vertical line indicates the genetic distance between flanking markers in centimorgan (cM). The marker interval which delimitates the position of the *Rpi-blb2* gene, based on detected recombination events in progeny clones, is indicated by a double arrow headed line.

Figure 12. Genetic complementation for late blight susceptibilty. Typical disease phenotypes of potato (*S. tuberosum*) leaves, 6 days after inoculation with a sporangiospore suspensions of *P. infestans* isolate 655-2A. Leaf derived from kanamycin resistant cv Kondor plants transformed with pBINPLUS (control; **A**), leaves derived from cv Kondor plants harbouring BAC SPB39 derived (**B**) or BAC 211 derived *RGC5* (**C**), leaf derived from kanamycin resistant cv Impala plants transformed with pBINPLUS (control; **D**), leaves derived from cv Impala plants harbouring BAC SPB39 derived (**E**) or BAC 211

derived *RGC5* (F). Panels A and D depict typical susceptible responses with extensive sporulating lesions of *P. infestans*. Panels B, C, E and F depict typical resistance reactions observed at the sites of inoculation on transgenic potato plants harbouring *Rpi-blb2*.

Figure 13. Nucleic acid sequences coding for the *Rpi-blb2* gene. **A.** Coding nucleic acid sequence of the *Rpi-blb2* gene. **B.** Coding nucleic acid sequence of the *Rpi-blb2* gene including the intron sequence (position 43-128). **C.** Sequence of the 7967 bp *Sau*3AI genomic DNA fragment of ARD 1197-16 BAC 211 present in p211F-C12, one of the two the genetic constructs used for genetic complementation for late blight resistance. The genomic fragment harbours the *Rpi-blb2* gene including natural regulatory elements necessary for correct expression of the gene. The initiation codon (ATG position 1546-1548) and the termination codon (TAG position 5433-5435) are underlined. **D.** Sequence of the 9949 bp *Sau*3AI genomic DNA fragment of *S. bulbocastanum* 2002 BAC BlbSP39 present in pSP39-20, one of the two the genetic constructs used for genetic complementation for late blight resistance. The genomic fragment harbours the *Rpi-blb2* gene including natural regulatory elements necessary for correct expression of the gene. The initiation codon (ATG position 1413-1415) and the termination codon (TAG position 5300-5303) are underlined.

Figure 14. Putative *Rpi-blb2* gene structure and deduced Rpi-blb2 protein sequence. **A.** Schematic representation of the *Rpi-blb2* gene structure. Horizontal lines indicate exons. Open boxes represent coding sequence. Lines angled downwards indicate the positions of intron sequences. **B.** Deduced Rpi-blb2 protein sequence. The amino acid sequence deduced from the DNA sequence of *Rpi-blb2* is divided into three domains (LZ, NBS and LRR). Hydrophobic residues in domain A that form the first residue of heptad repeats of the potential leucine zipoper (LZ) domain are underlined. Conserved motifs in R proteins are written in lowercase and in italic in the NBS domain. Residues matching the consensus of the cytoplasmic LRR are indicated in bold in the LRR domain. Dots in the sequence have been introduced to align the sequence to the consensus LRR sequence of cytoplasmic LRRs.

Figure 15. Alignment of the deduced protein products encoded by *Rpi-blb2*, *Mi-1.1* and *Mi-1.2*. The complete amino acid sequence of Rpi-blb2 is shown and amino acid residues from Mi-1.1 and Mi-1.2 that differ from the corresponding residue in Rpi-blb2. Dashes indicate gaps inserted to maintain optimal alignment. Amino acid residues that are specific for Rpi-blb2, when compared to those at corresponding positions in Mi-1.1 and Mi-1.2 are highlighted in bold and red. The regions of the LRRs that correspond to the β-strand/β-turn motif xxLxLxxxx are underlined. Conserved motifs in the NBS domain are indicated in lowercase. A vertical line indicates the division between CC-NBS

and LRR region. The position of the VLDL motif which is conserved in the third LRR of many plant R proteins but not in Rpi-blb2 is indicated by a shaded rectangle.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all refernces, patent applications, patents, and published patent applications cited throughout this application are hereby incorporated by reference.

Examples

Example 1: Evaluation of resistance in ABPT derived back cross clones and populations

BC2-clones ARF 87-507 and ARF 87-801 were selected from BC2-progeny obtained after two rounds of backcrossing on complex species hybrid ABPT-clone number 55 (Figure 1a) with late blight (LB) susceptible *S. tuberosum* cultivar Oberambacher Frühe as first parent and *S. tuberosum* cultivars Arkula (Figure 1b) and Blanka (Figure 1c) respectively as second parents. Similarly, BC2-clone ARF 87-601 was obtained by successive crossing on ABPT-clone 60 with LB susceptible *S. tuberosum* cultivars Alcmaria and Blanka (Figure 1d).

Clone ARF 87-601 was tested as part of a field test for screening of LB-resistance in the Toluca area in Mexico in 1991. A plot of clone ARF 87-601 with seven plants was evaluated in comparison to plots with nine plants each of the control cultivars Bildtstar, Eersteling and Pimpernel. According to the ratings for resistance to late blight in the Dutch National list of recommended potato cultivars of 1988, these control cultivars scored 3, 3 and 8 respectively on a scale from 3 to 8 of increasing resistance. Cultivar Pimpernel is considered as a source of partial resistance (Colon *et al.*, 1985). About forty days after planting, natural infection by *P. infestans* established. The development of LB in the foliage then was monitored eight times during the period from July 16th to September 2nd (Figure 2). There was a clear difference between the disease progress curves for ARF 87-601 in comparison to the control cultivars. At 74 days after planting, foliage of the control cultivars was completely or nearly completely blighted whereas clone ARF 87-601 showed no visible symptoms (Figure 2). Clones ARF 87-507, ARF 87-801 and again clone ARF 87-601 showed comparable results in a field test for screening of LB-resistance in the Benguet Province of the Philippines in 1992 (Figure 3). Ten plants each of the three BC2 clones, control cultivar Granola and the moderately LB resistant breeding clone AR 85-96-13, which was used as female parent to obtain AR 92-1197 (Figure 1d), were planted on August 25th. The percentage of blighted foliage was scored six times after occurrence of natural infection by *P. infestans*.

P. infestans. Disease progress curves of ABPT derived BC2-clones were markedly different when compared to cultivar Granola and clone AR 85-96-13 (Figure 3). BC2-clones showed no or little LB symptoms and no clear disease progress during the scoring period whereas cultivar Granola had almost completely blighted foliage at the third scoring date.

Clones ARF 87-601, ARF 87-507 and ARF 87-801 were used for further backcrossing with LB susceptible cultivars and breeding clones of *S. tuberosum* (Figure 1b to 1d). This breeding work resulted in four different mapping populations, tetraploid BC3-population ARG 95-15, tetraploid BC4-populations ARG 95-3 and ARP 96-11 and diploid BC4-population DP1. During the successive steps of this breeding work resistant clones ARF 87-507, ARF 87-601, ARF 87-801, AR 91-1263, AR 91-1292 and AR 92-1197 were selected on the basis of agronomic performance in common practice breeding evaluations as well as by screening their parents and relevant progenies in a field trial at Marknesse, the Netherlands, that was inoculated with the complex isolate IPO82001 of *P. infestans*. The diploid (2n=2x=24) clone ARD 1197-16 was selected among the progeny of cross AR 92-1197 x Phu 81-101 (Figure 1d), the latter parental clone being known for its capacity to induce parthenogenic seed set in the female parent (Hermsen and Verdenius, 1973). Initially, resistance to LB in ARD 1197-16 was found after repeated detached leaf assays using *P. infestans* isolates IPO82001, IPO655-2A and IPO428-2 and verified in a field trial in 1998 at Marknesse. The diploid status of clone ARD 1197-16 was confirmed by flow cytometry (Plant cytometry services, Schijndel, the Netherlands).

Clear segregation for the LB-resistance trait in ABPT-derived progeny and mapping populations was observed during successive years of field testing at the trial site of Marknesse, approximately 6 weeks after inoculation with isolate IPO82001 of *P. infestans*. Typically, resistant clones showed no or hardly any sporulating lesions whereas susceptible clones showed completely blighted foliage (Figure 4) In 2000, a total of 2851 clones from the mapping populations ARG 95-3 and ARP 96-11 were screened as single plant plots. On average, 24 percent of the clones showed phenotypes that could not unambiguously be classified as resistant or susceptible. Clones that could be classified as such showed segregation ratio's of resistant to susceptible phenotypes of 1 to 1 and 1 to 1.5 for populations ARG 95-3 and ARP 96-11, respectively (Table 2).

Detached leaf assay's with ABPT-derived progeny and mapping populations where found to be less accurate for phenotyping than screening under field conditions. Nevertheless, results of detached leaf assays were considered suitable for the initial determi-

nation of the phenotype of individual clones and thus, for construction of mapping populations.

Example 2: Genetic mapping of the *Rpi-blb2* resistance locus in ABPT derived back cross populations.

In all four mapping populations (Figure 1), resistance segregated as expected for a monogenic trait, suggesting the presence of a dominant resistance allele at a single locus (Table 2). This locus was designated the *Rpi-blb2* locus.

In order to identify markers linked to *Rpi-blb2*, an initial AFLP analysis with 14 primer combinations (pc) was carried out on DNA of 10 resistant and 10 susceptible ARG 95-15 progeny plants, based on detached leaf assay, including the parental clones. The testing of 21 potentially linked markers on an additional 89 plants identified several markers linked to resistance (Figure 5). Subsequent bulked segregant analysis (BSA) with 160 pc's on 2 resistant and 2 susceptible DNA pools, each containing genomic DNA of 8 resistant or susceptible ARG 95-15 progeny plants, respectively, identified a total of 58 AFLP markers potentially linked to resistance (Figure 5). When a number of these markers were tested on 137 progeny plants of ARG 95-3, they were also linked to resistance in this population, suggesting that the resistance in the two populations was determined by the same locus (Figure 6). These cosegregating markers mapped 3 to 28 centimorgan (cM) and 1 to 7.2 cM to one side of the locus in ARG 95-15 and ARG 95-3 respectively, suggesting that *Rpi-blb2* could be situated at a distal position on a chromosome.

To determine the position of the *Rpi-blb2* on the genetic map of potato, the two co-segregating AFLP markers E40M58 and E46M52 (Figure 6) were cloned into the pGEM-T vector (Promega, the Netherlands) and sequenced. Primers designed on the ends of the sequences of the cloned AFLP fragments (Table 3) were used to develop cleaved amplified polymorphic sequence (CAPS) marker E40M58 that was found to be cosegregating with the resistance trait in 25 resistant and 25 susceptible clones of ARG 95-3. CAPS marker E40M58 was subsequently tested on 46 progeny plants of the CxE mapping population (van Eck *et al.*, 1995). These data were added to the existing marker scores of the CxE population. Joinmap (Stam, 1993) linkage analyses mapped E40M58 8 cM distal to GP79 (Gebhardt *et al.*, 1991), positioning *Rpi-blb2* on the short arm of chromosome 6. In 178 progeny plants of population ARG 95-3 no recombination between *Rpi-blb2* and AFLP markers E40M58, E40M60 and CAPS marker CT119 was observed. AFLP marker E46M52 and sequence characterised amplified region (SCAR) marker CT216 mapped 2.2 cM proximal to the gene (Figure 7).

Example 3: Identification of a RGA marker linked to *Rpi-blb2*

In an attempt to identify functionally relevant markers linked to resistance, primers designed on the conserved motifs of the NBS domain of plant *R* genes (Leister *et al.*, 1996), were used in an adapted AFLP protocol (RGA-AFLP) to identify resistance gene analogue (RGA) specific markers.

Using the P-loop based primer S1 from Leister *et al.* (1996) in combination with the Eco00 AFLP primer, an RGA specific marker, S1E00 was developed which cosegregated with resistance and markers E40M58 and CT119 in the ARG 95-3 mapping population (Figure 6 and 7).

Example 4: Development of E40M58e and E46M52e SCAR markers for recombinant screening.

Using genomic DNA of AR 91-1263 as template, the cloned fragment of AFLP marker E46M52 was extended by TAIL-PCR. The primary TAIL-PCR was performed using primers ARO 77 (sp1) and ARO 94 (AD) Subsequently, the secondary PCR was performed using ARO 128 (sp2) and the tertiary PCR using ARO 129 (sp3) both in combination with primer AD. This resulted in an E46M52e fragment that was extended on the 5' end with approximately 500 bp. The E46M52e fragment was cloned in pGEM-T and sequenced. A new forward primer was designed on this sequence and PCR in combination with primer ARO 77 resulted in SCAR marker E46M52e that cosegregated with the resistant phenotype in the four *S. tuberosum* mapping populations and as CAPS marker also in population B6.

Using genomic DNA of ARD 1197-16 as template, the cloned fragment of AFLP marker E40M58 was also extended by TAIL-PCR. The primary TAIL-PCR was performed in both the 5' and 3' directions using sp1 primers ARO 73 (3') or 74 (5') in combination with primer AD. Subsequently, the secondary PCR was performed using as sp2 ARO 82 or 79, respectively. The fragments obtained from the secondary PCR, 750 bp from the 3' end and 400 bp from the 5' end were cloned in pGEM-T and sequenced. On the basis of both sequences, two new primers were designed resulting in a SCAR marker that cosegregated with resistance in mapping population ARG 95-3 and DP1 (Table 3). The fragment of SCAR marker E40M58e could be amplified in the resistant parents of mapping populations ARG 95-3 and DP1, which were both derived from ABPT clone 55 (Figure 1), but PCR amplification in the parents or progeny clones of mapping populations ARP 96-11 and ARG 95-15, which were both derived from ABPT clone 60, did not give any detectable PCR product. It was assumed that this could have been caused by minor differences in the genomic sequence and therefore, the AFLP

fragment was extended by TAIL-PCR using genomic DNA of clone AR 91-1292 as template. A fragment E40M58e2 of approximately 300 bp was obtained; cloned and sequenced. Comparison of the sequence with the original fragment of AFLP marker E40M58 showed that only the first 37 bp of the extended fragment were identical. PCR with primers designed on the sequence of E40M58e2 did not result in a polymorphic marker. Both of the extended markers E40M48e and E40M58e2 were tested on five resistant or susceptible clones of *S. bulbocastanum* (BGRC 8005 and 8006). Only the fragment of SCAR marker E40M58e could be amplified in four *S. bulbocastanum* clones, indicating that part of the sequence of E40M58e2 was not derived from *S. bulbocastanum*. This observation suggested that E40M58e was located on the border of the *S. bulbocastanum* introgression fragment in clone AR 91-1292 and that the position of the *Rpi-blb2* locus was proximal to marker E40M58e.

Example 5: Mapping of *Rpi-blb2* in a diploid mapping population derived from ABPT material

A total of 149 progeny clones of diploid mapping population DP1 were screened with markers E40M58e and E46M52e. No recombination was found between these markers suggesting suppressed recombination in the genomic region studied when compared to the tetraploid mapping population ARG 95-3 (Figure 7). A subset of 112 clones was screened for resistance to *P. infestans* isolates IPO82001, IPO655-2A and IPO428-2 in a partially repeated detached leaf assay. Eleven of the clones (11%) showed intermediate reactions and were classified as having unknown phenotypes. Another 51 and 50 clones were classified as resistant and susceptible respectively. Three progeny clones DP1-28, DP1-79 and DP1-81 were identified that were putatively recombined between the *Rpi-blb2* locus and the markers E40M58e and E46M52e. In 2000, a subset of 50 out of the 112 phenotyped clones was tested for resistance to LB in the field at the trial site of Marknesse. Conclusive results on the phenotype for LB resistance were obtained for 33 out of the 50 clones. The phenotype of clones 28 and 81 as determined with the detached leaf assay appeared to be erroneous. Thus, it was concluded that these clones did not represent recombination events between *Rpi-blb2* and the markers used. The phenotype of clone DP1-79 could not be verified conclusively under field conditions and this clone may represent the only recombination event between the *Rpi-blb2* locus and the markers E40M58e and E46M52e in 101 progeny clones of DP1 (1 cM). Since it was shown that two markers, linked to the resistance trait in ARG 95-15, ARG 95-3 and ARP 96-11, cosegregated with the same locus for LB-resistance in DP1, it was concluded that the DP1 parental clone ARD 1197-16 was suitable as a source for *Rpi-blb2* gene isolation in a map based cloning approach.

Example 6: Physical mapping of the ABPT derived *Rpi-blb2* locus

The resistant clone ARD 1197-16, heterozygous for the *Rpi-blb2* locus, was used as source DNA for the construction of a BAC library (hereafter referred to as the ARD 1197-16 BAC library). High molecular weight DNA preparation and BAC library construction were carried out as described in Rouppe van der Voort *et al.* (1999). Initially, a total of 67968 clones with an average insert size of 100 kb, which corresponds to approximately 7 genome equivalents, were individually stored in 177 384-well microtiter plates at -80°C. Marker screening of the ARD 1197-16 BAC library was carried out as described in Rouppe van der Voort *et al.* (1999). Essentially, DNA pools generated for each 384-well plate were screened by PCR with SCAR or CAPS markers linked to the *Rpi-blb2* locus in order to build a BAC contig across the *Rpi-blb2* locus.

Screening of the ARD 1197-16 BAC library with markers E40M58e, S1E00 and CT119 identified several positive BAC clones, which served as seed BACs from which a chromosome walk across the *Rpi-blb2* locus was initiated. Marker E40M58e was used to isolate the BAC clones 69 and 141 whereas BAC clones 14, 24, 123 and 133 were positive for marker S1E00. Marker CT119 was used to isolate BAC 67. After sequencing the left (L) and right (R) borders of these BAC clones, a new set of markers was developed; 14L, 24L, 24R, 69L, 69R, 141R, 123L, 123R, 133R and 67L. Screening of the isolated BAC clones with these markers showed that the following pairs of BAC clones shared overlap: the right side of 123 with the left side of 133, 14 completely with 24, and the left side of 69 with the right side of 141. BAC 67 did not share overlap with the other BAC clones. The finding that the S1E00 positive BAC clones 14, 24, 123, and 133 did not form a single contig indicated that S1E00 was a repetitive sequence. This, together with the finding that the right BAC-end sequences of BAC clones 24 and 123 showed high homology to different regions of the *Mi1* resistance gene from tomato (Milligan *et al.*, 1998, Simons *et al.*, 1998), suggested that the *Rpi-blb2* locus harboured more than one *RGA*. Screening of the initial ARD 1197-16 BAC library with markers 141R, 24L, 24R and 123L did not lead to contig extension. However, screening of the library with markers 123R and 133R resulted in the isolation of BAC clones 99 and 113, thereby extending the BAC 123/133 contig in one direction. BAC-end sequencing of these two BAC clones lead to the development of two new markers, 99L and 113R. Screening of the ARD 1197-16 BAC library with 69R lead to the extension of the 141/69 contig. Consecutive screening of the BAC library with markers derived from BAC clones that further extended this contig lead to the isolation of BAC clones 36, 41 and 112, and the development of markers 36L, 41L and 112L.

In an attempt to complete the BAC contig across the *Rpi-blb2* locus, the ARD 1197-16 BAC library was enlarged with an additional 38864 BAC clones of ~100kb (384-well

plate numbers 178-273). This second library was screened with markers 24L, 24R, 123L, and 141R, leading to the identification of BAC clones positive for both 24R and 123L (e.g. 191) and BAC clones positive for 24L (211, 242). In this way, the gap between BAC 24 and 123 was closed and the 24/14 contig was extended towards BAC clone 141. There were no new clones in the extended ARD 1197-16 library that were positive for marker 141R.

Example 7: Construction of additional markers in BAC 123/133 region.

In an attempt to develop additional polymorphic markers from BAC 123 and 133, a 10 kb sub-clone library was constructed of both BAC 123 and 133. BAC DNA was partially cleaved with *Sau3*AI and fragments of approximately 10 kbp were cloned in the *Bam*HI site of vector pBINPLUS. In order to select clones containing the original BAC-end sequence, 288 subclones of BAC 123 and 192 of BAC 133 were screened with the BAC-end markers 123L or 133R. In total 14 subclones were positive for marker 123L and 11 for marker 133R. Subsequently, the orientation of the BAC-end positive clones was determined by several PCRs using either the forward or reverse primer of the relevant BAC-end marker in combination with primers M13F or M13R (Table 3). For marker 123L three sub-clones and two sub-clones for marker 133R were selected and the ends not containing the 123L or 133R marker were sequenced (approximately 500 bp). Based on the new sequence two new primers were designed for subclone 123 resulting in marker 123L2 and two new primers were designed for subclone 133 resulting in marker 123R2. SCAR marker 123L2, which was located 10 kbp proximal to marker 123L, appeared to be polymorphic in mapping populations ARG 95-3, ARP 96-11 and as CAPS in B6. SCAR marker 133R2, which was located 10 kbp distal to marker 133R, was only polymorphic in mapping populations ARG 95-3 and ARP 96-11.

Example 8: Fine mapping of the *Rpi-blb2* locus in ABPT derived mapping populations.

In order to fine map the *Rpi-blb2* locus in ABPT derived mapping populations a total of 2283 new progeny clones of mapping population ARG 95-3 and 598 clones of mapping population ARP 96-11 were tested for resistance to LB in the field at the trial site of Marknesse in 2000 (Table 2). In population ARG 95-3 846 clones (37%) were scored susceptible and 886 clones resistant (39%). The phenotypes of the remaining 551 clones were unclear. In population ARP 96-11 256 clones (45%) were scored susceptible and 170 clones (30%) resistant. The phenotypes of the remaining 142 (25%) were unclear (Table 2). The 846 and 170 resistant clones from mapping populations ARG 95-3 and ARP 96-11, were selected for recombinant screening with SCAR marker CT216 and CAPS marker 41L or 36L, respectively. In total 85 (9.6 cM) and 22

(12.9 cM) recombinants were obtained in mapping populations ARG 95-3 and ARP 96-11 respectively, that were subsequently screened with CAPS marker 67L, reducing the number of recombinants to 5 (0.56 cM) in the marker interval 67L – 36L in case of mapping population ARG 95-3 and to 4 recombinants (2.35 cM) in the marker interval 67L – 41L in case of the mapping population ARP 96-11 (Figure 8). These remaining 9 recombinants were further analysed with SCAR and CAPS markers 113R, 99L, 133R, 133R2, 123R, 123L, 24R, 14L, 24L, 141R, 69L, E40M58e and 69R. The latter two markers were scored only in mapping population ARG 95-3.

In population ARG 95-3 two clones showed recombination between markers E40M58e and 69L, positioning the *Rpi-blb2* gene 0.23 cM proximal to marker E40M58e. Two other clones were recombined between markers 113R and 67L and one was recombined between markers 133R2 and 133R, positioning the *Rpi-blb2* gene 0.11 cM distal to marker 133R.

In population ARP 96-11, no recombination was detected between markers 41L and 69L, positioning the *Rpi-blb2* gene 0.58 cM proximal to marker 36L. Two progeny clones were recombined between markers 113R and 67L, and one clone was recombined between markers 99L and 133R, positioning the *Rpi-blb2* gene 0.58 cM distal to marker 99L (Figure 8; Figure 9).

Example 9: Evaluation and genetic mapping of late blight resistance in a *S. bulbocastanum* intraspecific mapping population.

In order to develop an intraspecific mapping population of *S. bulbocastanum*, a resistant clone Blb 2002 was obtained from an inter accession cross (Figure 10). This clone was reciprocally crossed with a susceptible clone Blb 48-5 that was selected also in progeny from an inter accession cross (Figure 10). The resulting population was designated B6 with synonyms B6a, Blb 99-229, Blb 00-7 and Blb 00-8.

Initially a small group of 47 progeny plants of the B6 population was screened for resistance to *P. infestans* in a partially repeated detached leaf assay using a sporangiospore solution of isolate IPO655-2A of *P. infestans* as inoculum. Plants with leaves that clearly showed sporulating lesions 6 to 9 days after inoculation were considered to have a susceptible phenotype whereas plants with leaves showing no visible symptoms or necrosis at the side of inoculation in the absence of clear sporulation were considered to be resistant. Of the 47 seedlings, 23 scored resistant and 24 susceptible. These data indicated that the progeny of mapping population B6 gave clear segregation of the resistance trait in the detached leaf assay and that resistance could be due to a single dominant gene or a tightly linked gene cluster. In order to determine the

chromosome position of this locus, 46 seedlings were analysed with markers 112L and E46M52e. Marker 112L was found to be linked in repulsion with the resistant phenotype, as only two recombinants were obtained between this marker and the phenotype of the 46 seedlings (4 cM). Also, marker E46M52e was found to be linked in repulsion with the resistant phenotype. Here, five recombinants were obtained between marker E46M52e and the phenotype (11 cM). Furthermore, markers 69R, 69L and 141R were used for analysis of the seven recombinants between markers 112L and E40M58e with an additional group of 6, 15 and 14 non recombined seedlings respectively, and found to be completely linked in either coupling (marker 69R) or repulsion phase (markers 69L and 141R) to resistance, indicating that the resistance gene was located at the same locus, i.e. *Rpi-blb2*, as in the ABPT-derived mapping populations.

In order to determine the position of *Rpi-blb2* more precisely relative to the available markers, another 849 seedlings of the B6 mapping population and 1054 seedlings from the reciprocal cross (Figure 10) were grown and analysed for recombination between the markers E46M52e and 112L. Thus, in addition to the initial 47 seedlings, a total of 1903 individual offspring clones of the B6 population were screened. Recombination between markers E46M52e and 112L was detected in a total of 138 of these seedlings (7,25 cM). Fine mapping of the *Rpi-blb2* locus was carried out in two steps. Firstly, the group of 138 recombinants was reduced to 19 by additional screening with markers 14Lb, 113R, 123L2, 24L, 141R and 69L (Table 3), derived from left (L) and right (R) border sequences of BAC clones isolated from the ARD 1197-16 BAC library and subsequent selection of all the seedlings that were recombined between markers 113R and 69L. Possibly due to double recombination, 4 recombinants gave patterns for the markers scored that deviated from scores expected in the case of single recombination events in the genetic interval studied and when assuming co-linearity of markers. These were withdrawn from further analyses. Secondly, the remaining 15 recombinants were analysed with markers from border sequences of BAC clones isolated from the Blb 2002 library, SPB39L and SPB30L, or with MiGA markers 24L9spec, 24Lspec and 14L24L (Table 3). Results of marker analyses of these remaining 15 recombinants, which gave clearly interpretable marker scores and phenotypes, positioned the *Rpi-blb2* locus between markers 69L and 24L, on a 0.11 cM (n=1899) genetic interval (Figure 11).

Example 10: *Mi*GA markers

Southern analysis of BAC clones 14, 24, 123 and 133 using markers 123R, 14L, or 24L as probes showed that these BAC clones contained several resistance gene analogs (RGAs). In view of the homology between the sequences of markers 14L, 24L and 123R with the *Mi1* gene from tomato, RGAs within the *Rpi-blb2* region are hereafter

referred to as *Mi* gene analogs (*Mi*GAs). In an attempt to develop additional polymorphic markers within the *Rpi-blb2* interval, PCR fragments generated from BAC clones 24 and 123 with the primer combination 14LR and 24LF were cloned into the pGEM-T vector (Promega, the Netherlands) and partially sequenced. Based on the alignment of these partial sequences, a set of universal primers were designed, univ14L and univ24L (Table 3), with the aim to amplify the corresponding region of as many as possible *Mi*GAs within the *Rpi-blb2* interval. This universal primer set was subsequently used to develop *Mi*GA specific SCAR/CAPS markers linked to *Rpi-blb2* (e.g. markers 14L24L, 24Lspec, 24L9spec; Figure 9).

Example 11: Physical mapping of the *S. bulbocastanum* derived *Rpi-blb2* locus.

The resistant clone Blb 2002 heterozygous for the *Rpi-blb2* locus, was used as source DNA for the construction of the *S. bulbocastanum* BAC library, hereafter referred to as the Blb 2002 BAC library. High molecular weight DNA preparation and BAC library construction were carried out as described previously. A total of approximately 100.000 clones were generated and stored as 50 bacterial pools containing approximately 2000 white colonies. These bacterial pools were generated by scraping the colonies from the agar plates into Luria Broth medium containing 18% glycerol and 12.5 µg/ml chloramphenicol using a sterile glass spreader. For the screening of the Blb 2002 BAC library, plasmid DNA was isolated from each pool of clones using the standard alkaline lysis protocol and PCR was carried out to identify positive pools. Bacteria corresponding to positive pools were diluted and plated on Luria Broth agar plates containing chloramphenicol (12.5 µg/ml). Individual white colonies were subsequently picked into 384-well microtiter plates and single positive BAC clones subsequently identified as described previously. Names of BAC clones isolated from the Blb 2002 BAC library carry the prefix BlbSP.

In order to build a Blb 2002 derived BAC contig across the *Rpi-blb2* genetic marker interval (69L-24L) the Blb 2002 BAC library was screened with markers 141R and 24L. This lead to the isolation of BAC clones BlbSP39 and BlbSP30, which overlap with each other and span the 141R-24L marker interval. BAC end sequences of both BAC clones were used to develop the markers SPB30L and SPB39L (Figure 9).

Example 12: Complementation analyses.

For complementation purposes, all *Rpi-blb2* gene candidates, i.e. all *Mi*GAs present on BAC clones BlbSP30, BlbSP39, 24, 242 and 211, were targeted for subcloning into the binary vector pBINPLUS (van Engelen *et al.*, 1996). This was done as follows. Aliquots of approximately 1 µg BAC DNA were digested with 1U, 0.1U or 0.01U of *Sau*3AI re-

striction enzym for 30 min. The partially digested BAC DNA was subjected to contour-clamped homogeneous electric field (CHEF) electrophoresis at 4°C in 0.5 X TBE using a linear increasing pulse time of 1-10 sec and a field strength of 6 V/cm for 16 hr. After electrophoresis, the agarose gel was stained with ethidium bromide to locate the region of the gel containing DNA fragments of approximately 10kbp in size. This region was excised from the gel and treated with GELASE (Epicentre Technologies, USA) according to the manufacturer. The size selected DNA was ligated to the *Bam*HI-digested and dephosphorylated binary vector pBINPLUS (van Engelen *et al.*, 1995) followed by transformation to ElectroMAX *E. coli* DH10B competent cells (Life Technologies, UK). Per BAC clone a total of 384 clones were PCR screened for the presence of *Mi*GA sequences using the primers univ24L and univ14L (Table 3). Positive clones were selected for further characterisation. Based on the restriction pattern of the 14L24L fragments digested with the enzymes *Rsa*I, *Taq*I, *Alu*I, *Dpn*II or *Mse*I, the different groups of *Mi*GAs were identified. The *Mi*GA harbouring the marker 24L, which was completely present on BAC clones BIbSP39, 211 and 242 was not detected with the universal primers univ14L and univ24L.

The relative position of the *MiGA* sequences in the 10kbp subclones was determined by PCR using internal primers 123Mi and 14L2 for the 5' end and univ14L and 24L2 for the 3' end in combination with primers derived from pBINPLUS vector sequences (ARO 295 and 296; Table 3). Two subclones per RGA of each BAC-library were selected for transformation.

For complementation analysis, the selected subclones were transferred to the susceptible potato cultivars Impala and Kondor through *Agrobacterium* mediated transformation using isolate UIA143 (Farrand *et al.*, 1989) or AGLO (Lazo et al., 1991). Primary transformants harbouring the transgenes of interest were tested for resistance to *P. infestans* in detached leaf assays using isolate IPO655-2A and IPO82001 (Table 4). Only the genetic constructs harbouring *RGC5*, both derived from *S. tuberosum* and *S. bulbocastanum*, were able to complement the susceptible phenotype both in cultivar Impala and in Kondor; in total 18 out of 19 *RGC5* containing primary transformants were resistant (Table 4, Figure 12) whereas all *RGC1*, *RGC2*, *RGC3*, *RGC4*, *RGC6* and *RGC7* containing primary transformants were susceptible to *P. infestans*. As the *RGC5* transformants showed similar resistance phenotypes as the resistant *S. bulbocastanum* parent of mapping population B6, *RGC5* was designated the *Rpi-blb2* gene. The homologues RGC8 and RGC24L can also be transferred to the described susceptible potato cultivars and tested for resistance to P. infestans in a detached leaf assay.

A selection of primary transformants containing *RGC5* was analysed for copy number by Southern analysis. *Eco*RI digested genomic DNA was hybridised with a *nptII* probe (Table 3). Based on the presence of the number of *nptII* hybridising fragments, the primary transformants contained at least 1 to 11 transgene inserts. In total, 4 single copy integrations in cultivar Impala and 6 in cultivar Kondor were observed of which one cultivar Kondor transformant appeared to have a *P. infestans* susceptible phenotype.

To investigate whether *Rpi-blb2* can also complement the susceptible phenotype in tomato, primary transformants of cultivar Moneymaker harbouring the *Rpi-blb2* gene construct can be produced and tested with a tomato isolate of *P. infestans* or with the potato derived isolates IPO82001 and IPO655-2A. The disease resistance assay can reveal that *Rpi-blb2* is able to complement a susceptible tomato phenotype.

Example 13: Rpi-blb2 gene structure and putative amino acid sequence

The inserts of the *RGA5* containing binary subclones 211F/C12 and SP39-20 were sequenced by a primer walk strategy whereby consecutive rounds of sequencing were carried out using a set of nested primers which were designed as the contiguous sequence was extended. The first set of sequences was generated using the M13F and M13R primers. The complete sequences of the inserts of clones 211F/C12 and SP39-20 consisted of 7967 and 9949 nucleotides (nt), respectively (Figure 13). The sequence of clone 211F/C12 was identical to the corresponding sequence within clone SP39-20. The position and putative structure of *Rpi-blb2* was predicted using GENSCAN (Burge and Karlin, 1997), GeneMark (Lukashin and Borodovsky 1998) and through alignment to the gene sequences of *Mi1.1* and *Mi1.2*.

The *Rpi-blb2* gene putatively contains two introns. Based on homology of these intron sequences to those present in *Mi1.1* and *Mi1.2* we predict intron 1 to be positioned within the 5' untranslated region (UTR), ending 32 nucleotides upstream of the putative ATG start codon. Intron 2 is predicted to be 86 nt long starting 43 nucleotides downstream of the ATG start codon of the gene (Figure 13). The coding sequence of the *Rpi-blb* transcript is predicted to be 3804 nt. The exact length of the transcript can be determined through 5'and 3' rapid amplification of cDNA ends (RACE) using the GeneRacer™ kit (Invitrogen™, Groningen, the Netherlands). 3' RACE is carried out with a set of nested *Rpi-blb2* specific oligonucleotides, which match sequences within the last 500 nt of the coding sequence of *Rpi-blb2* in combination with the GeneRacer™ 3' primer and GeneRacer™ 3' nested primers. 5' RACE is carried out on cDNA synthesised with an oligodT primer or a primer complementary to a *Rpi-blb2* specific sequence 500-1000 nt downstream of the ATG codon, using a set of nested *Rpi-blb2* specific oligonucleotides which are complementary to sequences within first 500 nt of

the coding sequence of *Rpi-blb2* in combination with the GeneRacer™ 5' primer and GeneRacer™ 5' nested primer.

The deduced open reading frame of the *Rpi-blb2* gene encodes a predicted polypeptide of 1267 amino acids with an estimated molecular weight of 146 kDa (Figure 14). Several functional motifs present in *R* genes of the NBS-LRR class of plant *R* genes are apparent in the encoded protein. As illustrated in Figure 14, the Rpi-blb2 protein belongs to the leucine zipper (LZ) subset of NBS-LRR resistance proteins. The N-terminal half of the Rpi-blb2 protein contains a potential LZ region between amino acids 413 and 434 and six conserved motifs indicative of a nucleotide-binding site (van der Biezen and Jones, 1998). The C-terminal half of Rpi-blb comprises a series of 15 irregular LRRs that can be aligned according to the consensus sequence hxxhxxLxxLxLxxC/N/Sx(x)LxxLPxx observed in other cytoplasmic R proteins, whereby h can be L, I, M, V or F, and x any amino acid residue (Jones and Jones; 1997).

Example 14: Homology to known state of the art R gene sequences

To identify *in silico* homologues of the *Rpi-blb2* gene, BLAST searches (Altschul *et al.*, 1990) were carried out with the coding sequence of the *Rpi-blb2* gene. BLASTN searches identified a number of sequences with significant homology to the *Rpi-blb2* gene. Using the alignment programme ClustalW (standard settings) in the DNAStar software package, we determined that the *Rpi-blb2* coding sequence shares the highest homology to *Mi-1.1* (89.8%) and *Mi-1.2* (89.7%) (Genbank accession numbers AF039681 and AF039682, respectively). The latter sequence corresponds to the *Mi* gene from tomato that confers resistance to three of the most damaging species of the root knot nematodes (*Meloidogyne* spp.) (Milligan *et al.*, 1998). In addition nucleotides 2410-3461 of the *Rpi-blb2* coding sequence share 87.8% sequence homology to a partial NBS-LRR sequence from *Solanum nigrum* (Genbank accession number AY055116.1). At the amino acid level the putative Rpi-blb2 protein sequence shares the highest homology to Mi-1.1 (82% identity) and Mi-1.2 (81% identity) (Genbank accession numbers AF039681 and AF039682).

Through ClustalW alignment of the deduced amino acid sequences of Rpi-blb2, Mi-1.1 and Mi-1.2 we have identified 200 amino acid (aa) residues which are unique to Rpi-blb2 (Figure 15). Of these, 31 are found at hypervariable positions, i.e. the residue at this position is different in all three sequences and 11 are encoded by small insertions (one 3 aa residue insertion and one 8 aa residue insertion). The rest are Rpi-blb2 specific in that the aa residues encountered at corresponding positions in Mi-1.1 and Mi-1.2 are different from the Rpi-blb2 residue but conserved in the two Mi protein sequences (Figure 15). Interestingly, the VLDL motif that is conserved in the third LRR of

many NBS-LRR proteins including Mi (Axtell *et al.*, 2001; Banerjee *et al.*, 2001), is not conserved in Rpi-blb2 (Figure 15).

Example 15: *Rpi-blb2* allele mining in wild *Solanum* species

Using primers ARF1F and ARF1R (Table 3B), designed around the start and stop codon of the *Rpi-blb2* gene, it is possible to amplify by PCR, alleles of *Rpi-blb2* from any *Solanum* species. The amplification products can be cloned between transcriptional regulatory sequences in a binary plasmid and transferred to *S. tuberosum* through *Agrobacterium* mediated transformation or any method known to those skilled in the art. The resulting primary transformants can subsequently be analysed for resistance to *P. infestans* or to any pathogen for which potato is a host plant.

Example 16: Material and methods

Plant material and development of mapping populations in (1) Solanum tuberosum. Complex interspecifc hybrid clones, designated ABPT, were made by Hermsen and co-workers (Hermsen, 1966; Hermsen and Ramanna, 1969; Ramanna and Hermsen, 1971; Hermsen and Ramanna, 1973; Hermsen, 1983; Hermsen, 1994) (Figure 1a). The chromosome doubling step with colchicines was described by Hermsen (1966) and Hermsen and De Boer (1971). The resistance in some of the ABPT clones to *P. infestans* is believed to be derived from either one or both of the accessions from *S. bulbocastanum* BGRC 8007 (CGN 21306; Pi 275196) and BGRC 8008 (CGN 17693; Pi 275198) that were used in the initial cross to produce hybrids between *S. acaule* and *S. bulbocastanum*, since all other parents that were used in the breeding scheme for ABPT-clones were susceptible or only partially resistant to *P. infestans* in detached leaf assays (Hermsen and Ramanna, 1973). Tubers from 19 clones of population [(ABPT clone number 55 x cultivar (cv) Oberambacher Frühe) x cv Arkula], from 7 clones of population [(ABPT clone number 55 x cv Oberambacher Frühe) x cv Blanka] and from 5 clones of population [(ABPT clone number 60 x cv Alcmaria) x cv Blanka] were received in 1988 from the former Department of Plant Breeding of the Wageningen Agricultural University (Wageningen, the Netherlands). Clones ARF 87-507, ARF 87-801 and ARF 87-601 were selected from these populations respectively. They represented offspring from a second backcross (BC2) with the complex interspecific ABPT-clones and were used for further back crosses that resulted in one tetraploid BC3 population, two tetraploid BC4 populations and one diploid BC4 population that were used for genetic mapping of the *Rpi-blb2* gene (Figure 1). The tetraploid *Solanum tuberosum* mapping population ARG 95-15 was produced by crossing *P. infestans* resistant clone ARF 87-507 with the susceptible cultivar Alkon. Tetraploid population

ARG 95-3 was produced by crossing *P. infestans* resistant clone AR 91-1263 with the susceptible cultivar Cosmos. Tetraploid population ARP 96-11 was produced by crossing resistant clone AR 92-1292 with the susceptible cultivar Celeste. The diploid population DP1 was obtained by crossing the resistant clone ARD 1197-16 with the susceptible clone ARD 93-2090 (Figure 1).

Plant material and development of mapping populations in (2) Solanum bulbocastanum.

The diploid *S. bulbocastanum* mapping population, designated B6 (synonym B6a, Blb 99-229, Blb 00-7 and Blb 00-8), was developed by crossing a *P. infestans* resistant clone Blb 2002 (synonym M94-81-C) with a susceptible clone Blb 48-5. Results from reciprocal crosses of population B6 were combined. The resistant parental clone of population B6 was obtained from a cross between *S. bulbocastanum* clone Blb 93-D26-3 (accession BGRC 8002; CGN 17690; PI 275187) as female parent and *S. bulbocastanum* clone Blb 93-60-10 (accession BGRC 8006; PI 275194) as male parent. The susceptible parental clone of population B6 was obtained from a cross between *S. bulbocastanum* clones from accessions BGRC 8005 (CGN 17692, PI 275193) and BGRC 8006 (Figure 2).

Disease assays;(1) Phytophthora infestans *isolates*
Three different *P. infestans* isolates were obtained from Plant Research International B.V. (Wageningen, the Netherlands). Isolates had different race structures and mating types as follows: IPO82001: race structure 1.2.3.4.5.6.7.10.11, mating type A2; IPO655-2A: race structure 1.2.3.4.5.6.7.8.9.10.11, mating type A1; IPO428-2: race structure 1.2.3.4.5.6.7.8.9.10.11, mating type A2 (Flier *et al.*, 2003).

Disease assays; (2) field trials
Glasshouse grown seedling tubers or field grown seed potatoes were planted at trial sites in Marknesse, the Netherlands from 1985 tot 2002, in the Toluca area of Mexico in 1991 or at a site in the Benguet Province in the Philippines in 1992. For individual clones, plots were planted consisting of 1 to 10 tubers. Approximately 8 weeks after planting, the field at Marknesse was inoculated with a sporangiospore solution of *P. infestans* isolate IPO82001 and disease scores were collected 3 to 6 weeks after inoculation. Clones that were free or nearly free from late blight were classified as having a resistant phenotype whereas clones with a complete of nearly complete blighted foliage were classified as susceptible. Clones with intermediate reactions to late blight were classified as having an unknown phenotype. At the field trials in Mexico and the Philippines, natural infection had to occur. Once this natural infection by *P. infestans* established, the percentage of blighted foliage of plants on each plot was scored on 8

and 6 days respectively on a 1-9 scale were estimated percentages of blighted foliage from 1 tot 9 were: 0, 3, 10, 25, 50, 75, 90, 97 and 100 (Estrada-Ramos *et al.*, 1983).

Disease assays; (3) detached leaves

For the detached leaf assay, leaves from plants grown for 6 to 12 weeks in the greenhouse were placed in pieces of water-saturated florists foam, approximately 35x4x4 cm, and put in a tray (40 cm width, 60 cm length and 6 cm height) with a perforated bottom. Each leaf was inoculated with two droplets (25 µl each) of sporangiospore solution on the abaxial side. Subsequently, the tray was placed in a plastic bag on top of a tray, in which a water-saturated filter paper was placed, and incubated in a climate room at 17ºC and a 16h/8h day/night photoperiod with fluorescent light (Philips TLD50W/84HF and OSRAM L58W/21-840). After 6 to 9days, the leaves were evaluated for the development of *P. infestans* disease symptoms.

Evaluation:

Plants with leaves that clearly showed sporulating lesions 6 to 9 days after inoculation were considered to have a susceptible phenotype, whereas plants with leaves showing no visible symptoms or necrosis at the side of inoculation in the absence of clear sporulation were considered to be resistant.

Plant DNA marker screening

Genomic DNA was extracted from young leaves according to Bendahmane *et al.* (1997). For PCR analysis, 15 µl reaction mixtures were prepared containing 0.5 µg DNA, 15 ng of each primer, 0.2 mM of each dNTP, 0.6 units Taq-polymerase (15 U/µl, SphaeroQ, Leiden, the Netherlands), 10 mM Tris-HCl pH 9, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100 and 0.01% (w/v) gelatin. The PCRs were performed using the following cycle profile: 25 seconds DNA denaturation at 94ºC, 30 seconds annealing and 40 seconds elongation at 72ºC. As a first step in PCR-amplification DNA was denatured for 5 min at 94ºC and finalised by an extra 5 min elongation step at 72ºC. The amplification reactions were performed in a Biometra® T-Gradient or Biometra® Uno-II thermocycler (Westburg, Leusden, the Netherlands). Depending on the marker, the PCR product was digested with an appropriate restriction enzyme. An overview of the markers including primer sequences, annealing temperature and restriction enzymes if appropriate, is given in Table 3. Subsequently, the (cleaved) PCR products were analysed by electrophoresis in agarose or acrylamide gels. For acrylamide gel analysis, the CleanGel DNA Analysis Kit and DNA Silver Staining Kit (Amersham Pharmacia Biotech Benelux, Roosendaal, the Netherlands) were used.

Elongation of AFLP fragments by Thermal asymmetric interlaced (TAIL)-PCR

Elongation of the sequence of an AFLP fragment was performed by TAIL-PCR according to Liu and Whittier (1995). Shortly, elongation of AFLP fragments was performed

using 2 or 3 nested specific primers (sp) in combination with an arbitrary degenerate (AD) primer. The first PCR was performed with primers sp1 and AD, the second with sp2 and AD and the third with sp3 and AD according to the scheme described in Table 5. The PCR was performed in 25 µl reactions containing the standard PCR mix as described before, except that 30 ng of primer AD was used. The elongated fragments were cloned in pGEM-T (Promega, the Netherlands) and sequenced.

BAC library construction and screening

The resistant clone ARD 1197-16, heterozygous for the *Rpi-blb2* locus, was used as source DNA for the construction of the *S tuberosum* BAC library. The resistant clone Blb 2002 heterozygous for the *Rpi-blb2* locus, was used as source DNA for the construction of the *S. bulbocastanum* BAC library. High molecular weight DNA preparation and BAC library construction were carried out as described in Rouppe van der Voort *et al.* (1999). For the *S. tuberosum* BAC library, approximately 120.000 clones with an average insert size of 100 kb, which corresponds to 8 to10 genome equivalents were finally obtained. A total of approximately 70.000 clones were individually stored in 177 384-well microtiter plates at -80°C. Another 50.000 clones were stored as 14 bacterial pools containing approximately 4000 white colonies. These were generated by scraping the colonies from the agar plates into Luria Broth medium containing 18% glycerol and 12.5 µg/ml chloramphenicol using a sterile glass spreader. These so-called super pools were also stored at -80°C. Finally, another 37.000 clones were added to the *S. tuberosum* BAC library. The *S. bulbocastanum* BAC library consisted of 48 super pools of approximately 2.000 colonies.

Marker screening of the BAC library harbouring the individually stored BAC clones was carried out as described in Rouppe van der Voort *et al.* (1999). For the screening of the BAC library stored as super pools, plasmid DNA was isolated from each pool of clones using the standard alkaline lysis protocol and PCR was carried out to identify positive pools. Bacteria corresponding to positive pools were diluted and plated on Luria Broth agar plates containing chloramphenicol (12.5 µg/ml) Individual white colonies were subsequently picked into 384-well microtiter plates and single positive BAC clones subsequently identified as described above. Names of BAC clones isolated from the super pools carry the prefix SP (e.g. SP39).

Subcloning of candidate genes

Candidate RGAs were subcloned from BAC clone 24, 211, 242, BLBSP39 and BLBSP30 as follows. Aliquots of approximately 1 µg BAC DNA were digested with 1U, 0.1U or 0.01U of *Sau3A*I restriction enzym for 30 min. The partially cleaved BAC DNA was subjected to CHEF electrophoresis at 4°C in 0.5 X TBE using a linear increasing pulse time of 1-10 sec and a field strength of 6 V/cm for 16 hr. After electrophoresis.

the agarose gel was stained with ethidium bromide to locate the region of the gel containing DNA fragments of approximately 10 kbp in size. This region was excised from the gel and treated with GELASE (Epicentre Technologies, USA) according to the manufacturer. The size selected DNA was ligated to the *Bam*HI-cleaved and dephosphorylated binary vector pBINPLUS (van Engelen *et al.*, 1995) followed by transformation to ElectroMAX *E. coli* DH10B competent cells (Life Technologies, UK). A total of 192 clones were PCR screened for the presence of RGC sequences using the primers of marker 24L14L (Table 3). Positive clones were selected for further characterisation. Identification of clones harbouring *RGC1, RGC2, RGC3, RG4, RGC5, RGC6, RGC7, RGC8 and RGC24L* was carried out by sequencing 14L24L PCR fragments derived from positive clones. The relative position of the RGAs within a subclone was determined by PCR analysis using internal primers (24L2, 123Mi) in combination with pBINPLUS specific primers (Table 3).

Agrobacterium tumefaciens mediated transformation of potato

Binary plasmids harbouring the candidate genes were transformed to *A. tumefaciens* strains AGL0 (Lazo *et al.*, 1991) or UIA143 (Farrand *et al.*, 1989), the latter containing the helper plasmid pCH32 (Hamilton *et al.*, 1996). Overnight cultures of the transformed *A. tumefaciens* strains were used to transform potato tuber discs (cvs Impala and Kondor) according to standard protocols (Hoekema *et al.*, 1989; Fillati *et al.*, 1987). Shortly, certified seed potatoes of cultivars Impala and Kondor were peeled and surface sterilised for 30 min in a 1% sodium hypochlorate solution containing 0.1% Tween-20. Tubers were then washed thoroughly in large volumes of sterile distilled water (4 times, 10 min). Discs of approximately 2 mm thickness and 7 mm in diameter were sliced from cylinders of tuber tissue prepared with a corkbore. The tuber discs were transferred into liquid MS30 medium containing *A. tumefaciens* and incubated for 15 min. After removing the *A. tumefaciens* solution, the tuber discs were transferred to regeneration medium containing MS30, 0.9 mg/l IAA, 3.6 mg/l zeatine riboside and 8 g/l agar (Hoekema *et al.*, 1989). The plates were incubated at 24°C, 16 hour day-length (Philips TLD50W/84HF). After 48 hours of co-cultivation, the tuber discs were rinsed for 5 min in liquid MS medium including antibiotics, 200 mg/l vancomycin, 250 mg/l cefotaxim and 75 mg/l kanamycin, and transferred to regeneration medium supplemented with the same antibiotics. The plates were incubated at 24°C, 16 hour day-length (Philips TLD50W/84HF). Every three weeks, the tuber discs were transferred to fresh medium. Regenerating shoots were transferred to MS30 medium containing 75 mg/l kanamycin. Rooting shoots were propagated *in vitro* and tested for absence of *A. tumefaciens* cells by incubating a piece of stem in 3 ml Luria Broth medium (3 weeks, 37°C, 400 rpm). One plant of each transformed regenerant was transferred to the greenhouse.

Agrobacterium tumefaciens mediated transformation of tomato

Seeds of the susceptible tomato line Moneymaker are rinsed in 70% ethanol to dissolve the seed coat and washed with sterile water. Subsequently, the seeds are surface-sterilised in 1.5% sodium hypochlorite for 15 minutes, rinsed three times in sterile water and placed in containers containing 140 ml MS medium pH 6.0 (Murashige and Skoog, 1962) supplemented with 10 g/l sucrose (MS10) and 160 ml vermiculite. The seeds are left to germinate for 8 days at 25ºC and 0.5 W/M^2 light.

Eight day old cotyledon explants are pre-cultured for 24 hours in Petri dishes containing a two week old feeder layer of tobacco suspension cells plated on co-cultivation medium (MS30 pH 5.8 supplemented with Nitsch vitamines (Duchefa Biochemie BV, Haarlem, the Netherlands), 0.5 g/l MES buffer and 8 g/l Daichin agar).

Overnight cultures of *A. tumefaciens* are centrifuged and the pellet is resuspended in cell suspension medium (MS30 pH 5.8 supplemented with Nitsch vitamines, 0.5 g/l MES buffer, pH 5.8) containing 200 µM acetosyringone to a final O.D.$_{600}$ of 0.25. The explants are then infected with the diluted overnight culture of *A. tumefaciens* UIA143 containing pBINRGC5 for 25 minutes, blotted dry on sterile filter paper and co-cultured for 48 hours on the original feeder layer plates. Culture conditions are as described above.

Following the co-cultivation, the cotyledons explants are transferred to Petri dishes with selective shoot inducing medium (MS pH 5.8 supplemented with 10 g/l glucose, including Nitsch vitamines, 0.5 g/l MES buffer, 5 g/l agargel, 2 mg/l zeatine riboside, 400 mg/l carbenicilline, 100 mg/l kanamicine, 0.1 mg/l IAA) and cultured at 25ºC with 3-5 W/m^2 light. The explants are sub-cultured every 3 weeks onto fresh medium. Emerging shoots are dissected from the underlying callus and transferred to containers with selective root inducing medium (MS10 pH 5.8 supplemented with Nitsch vitamines, 0.5 g/l MES buffer, 5 g/l agargel, 0.25 mg/l IBA, 200 mg/l carbenicillin and 100 mg/l kanamycine).

RNA extraction

Total RNA can be isolated using Trizol® according to the protocol supplied by the manufacturer (Invitrogen™, Groningen, the Netherlands) with minor modifications. Briefly, 0.5 g of young leaf tissue is ground in liquid nitrogen and the powder suspended in 5 ml Trizol®. After a 5 min incubation at room temperature (RT), 0.5 ml chloroform is added, the suspension is vortexed and incubated for 2 min. After centrifugation (15 min, 11404 x g, 4ºC) the supernatant is transferred to a new tube and 2.5 ml isopropanol is added. After 10 min at RT, nucleic acids is precipitated (10 min, 11404 x g, 4ºC). The pellet is washed with 5 ml 70% ethanol (5 min, RT) and after cen-

trifugation (5 min, 6415 x g, 4ºC), the pellet iss dried and resuspended in 100 µl sterile distilled water.
PolyA RNA can be extracted from total RNA using the Oligotex™ kit (Qiagen, GmbH, Germany).

Rapid amplification of cDNA ends
The 5' and 3' ends of the *Rpi-blb2* cDNA and confirmation of putative intron positions can be determined by rapid amplification of cDNA ends (RACE) using the GeneRacer™ kit (Invitrogen™, Groningen, The Netherlands). 3' RACE is carried out with a set of nested *Rpi-blb2* specific oligonucleotides which match sequences within the last 500 nt of the coding sequence of *Rpi-blb2* in combination with the GeneRacer™ 3' primer and GeneRacer™ 3' nested primers. 5' RACE is carried out on cDNA synthesised with an oligodT primer or a primer complementary to a *Rpi-blb2* specific sequence 500-1000 nt downstream of the ATG codon, using a set of nested *Rpi-blb2* specific oligonucleotides which are complementary to sequences within first 500 nt of the coding sequence of *Rpi-blb2* in combination with the GeneRacer™ 5' primer and GeneRacer™ 5' nested primer.

AFLP fingerprinting and cloning of AFLP fragments
Template preparation and AFLP fingerprinting were essentially performed as described in Vos *et al.* (1995). In order to clone specific fragments ^{33}P-labelled AFLP fragments were excised out of the acrylamide gel by overlaying the polyacrylamide gels, dried on Whatmann 3MM paper, with autoradiogram images. The pieces of gel/paper underneath the band of interest were cut out and transferred to 200 µl of TE and incubated for 1 h at room temperature. Five microlitres of supernatant was used to re-amplify the fragment, using a PCR in which the *Eco*RI+0 in combination with *Mse*I+0 were used as primers. The re-amplified AFLP fragment was subsequently cloned into the pGEM-T cloning vector (Promega, the Netherlands) and the inserts of several clones sequenced.

The DNA sequence of the excised AFLP band was used to design locus-specific primers. The amplification product obtained with such primers was screened for internal polymorphisms with restriction enzymes. After restriction, the fragments were separated on a 2-3% agarose gel including ethidiumbromide.

RGA-AFLP analysis
Template preparation was essentially performed as described in Vos *et al.* (1995). However, the second amplification step was carried out with the P-loop based primer S1 from Leister *et al.* (1996) in combination with the *Eco*RI+0 AFLP primer. A 10 µl reaction mixture [0.5 µl ^{33}P-labelled S1 primer (10 ng/µl); 0.5 µl *Eco*R1+0 primer

(10 ng/µl); 0.8 µl dNTPs (5mM); 2 µl 10xGoldstar™ PCR buffer (Eurogenetc, Belgium); 1.2 µl $MgCl_2$ (25.mM); 0.06 µl Goldstar™ DNA polymerase (5U/µl) (Eurogentec, Belgium); 14.94 µl MQ water] was added to 10 µl diluted template (20x diluted in MQ water) and a PCR reaction performed using the following cycle profile: 45 seconds DNA denaturation at 94ºC, 45 seconds primer annealing at 49ºC and 2 min elongation step at 72ºC (35 cycles). Prior to the cycling the template DNA was denatured for 2 min at 94ºC and the PCR was finalised by a applying an extra 5 min elongation step at 72ºC. The amplification reactions were performed in a Perkin Elmer 9600 thermocycler. The labelled PCR products fragments were separated on a 6% polyacrylamide gel and the individual bands visualized by autoradiography according to standard procedures.

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Claims

1. A method for generating or increasing the resistance of a plant to a plant pathogen of the phylum Oomyceta comprising increasing the activity of Rpi-blb2 protein in the plant or a tissue, organ or cell of a plant or a part thereof.

2. The method of claim 1, wherein said Rpi-blb2 protein is encoded by a polynucleotide comprising a nucleic acid molecule selected from the group consisting of:

(a) nucleic acid molecule encoding at least the mature form of the polypeptide depicted in SEQ ID NO: 2 or 4;
(b) nucleic acid molecule comprising the coding sequence as depicted in SEQ ID NO: 1 or 3 or 5 or 6 encoding at least the mature form of the polypeptide;
(c) nucleic acid molecules the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b);
(d) nucleic acid molecule encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) to (c) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) to (c):
(e) nucleic acid molecule encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a) or (b);
(f) nucleic acid molecule comprising a fragment or a epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);
(g) nucleic acid molecule comprising a polynucleotide having a sequence of a nucleic acid molecule amplified from a nucleic acid library using a primer as listed in Tab. 3b;
(h) nucleic acid molecule encoding a fragment beginning with amino acid: 1, 30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1276, 1000, 500, 300, 200, 50, or 1 of a polypeptide encoded by any one of (a) to (g);
(i) nucleic acid molecule comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (d);
(j) nucleic acid molecule encoding a polypeptide being recognized by a monoclonal antibody that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (h);
(k) nucleic acid molecule obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (j) or of a fragment thereof of at least 20; and

(l) nucleic acid molecule the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);

or the complementary strand of any one of (a) to (l);
or expressing a polypeptide encoded by a segment of chromosome or linkage group 6 of Solanum bulbocastanum or Solanum tuberosum which co-segregates with a marker selected from table 3a or 3b and which mediates resistance to a pathogen of the phylum Oomycota
and whereby the polynucleotide has not the sequence of Mi1.1 or Mi1.2 as depicted in Seq. ID NO.: 7 or 9.

3. The method of claim 1 or 2, wherein the activity of a further resistance protein is increased.

4. The method of any one of claims 1 to 3, wherein activity is increased due to a de novo-expression.

5. The method of any one of claims 1 to 4, wherein the endogenous activity of a Rpi-blb2 and/or the further resistance protein is increased.

6. The method of any one of claim 1 to 5, comprising one or more of the following steps

a) stabilizing the resistance protein;
b) stabilizing the resistance protein encoding mRNA;
c) increasing the specific activity of the resistance protein;
d) expressing or increasing the expression of a homologous or artificial transcription factor for resistance protein expression;
e) stimulate resistance protein activity through exogenous inducing factors;
f) expressing a transgenic resistance protein encoding gene; and/or
g) increasing the copy number of the resistance protein encoding gene.

7. The method of any one of claims 1 to 6 which results in reduction in the sporulation index of at least 30% after infection with P. infestans compared to a wild type.

8. A polynucleotide encoding a Rpi-blb2 protein comprising a nucleic acid molecule selected from the group consisting of:

(a) nucleic acid molecule encoding at least the mature form of the polypeptide depicted in SEQ ID NO: 2 or 4;

(b) nucleic acid molecules comprising the coding sequence as depicted in SEQ ID NO: 1 or 3 or 5 or 6 encoding at least the mature form of the polypeptide;

(c) nucleic acid molecule the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b);

(d) nucleic acid molecule encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) to (c) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) to (c);

(e) nucleic acid molecule encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a) or (b);

(f) nucleic acid molecules comprising a fragment or a epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);

(g) nucleic acid molecule comprising a polynucleotide having a sequence of a nucleic acid molecule amplified from a nucleic acid library using the primers as listed in Tab.3b;

(h) nucleic acid molecule encoding polypeptide fragment beginning with amino acid: 1, 30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1276, 1000, 500, 300, 200, 50, or 30 of a polypeptide encoded by any one of (a) to (g);

(i) nucleic acid molecule comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (d);

(j) nucleic acid molecule encoding a polypeptide being recognized by a monoclonal antibodies that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (h);

(k) nucleic acid molecule obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (j) or of a fragment thereof of at least 20; and

(l) nucleic acid molecule the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);

or the complementary strand of any one of (a) to (l);

or encoding a polypeptide encoded by a segment of chromosome or of linkage group 6 of Solanum bulbocastanum or Solanum tuberosum which co-segregates with a marker selected from table 3a or 3b or comprises a replication site or

hybridisation site for said marker and which mediates resistance to pathogens of the phylum Oomyceta;
and whereby the polynucleotide has not the sequence of Mi1.1 or Mi1.2 as depicted in Seq. ID NO.: 7 or 9.

9. The polynucleotide of claim 8 or the method of any one of claims 1 to 7, wherein the marker is E40M58, CT119, or CT216.

10. The polynucleotide of claim 8 to 9 which is DNA or RNA.

11. A method for making a recombinant vector comprising inserting the polynucleotide of any one of claims 8 to 10 into a vector or inserting said polynucleotide and a further resistance protein.

12. A vector containing the polynucleotide of any one of claims 8 to 10 or comprising said polynucleotide and a further resistance gene or being produced by the method of claim 11.

13. The vector of claim 12 or the method of any one of claims 1 to 7 in which a polynucleotide encoding Rpi-blb2 protein or encoding the further resistance protein is operatively linked to expression control sequences and/or is operatively linked to a nucleic acid sequence encoding a transgenic expression regulating signal allowing expression in prokaryotic or eukaryotic host cells.

14. The vector of claim 12 or 13 or the method of any one of claims 1 to 7 in which the polynucleotide encoding Rpi-blb2 protein or encoding a further resistance protein is operatively linked to expression control sequences of the same species origin as the polynucleotide encoding Rpi-blb2 protein or the further resistance protein.

15. A method of making a recombinant host cell comprising introducing the vector of any one of claims 12 to 14 or introducing said vector and a vector for expressing a further resistance protein into a host cell.

16. A host cell produced according to the method of claim 15 or genetically engineered with the polynucleotide of any one of claims 8 to 10 or the vector of any one of claims 12 to 14 or genetically engineered with said vector or polynucleotide and a vector or a polynucleotide for expressing a further resistance protein.

17. The host cell of claim 16, which is E. coli, Baculovirus, Agrobacterium, or a plant cell.

18. A process for the production of a Rpi-blb2-polypeptide comprising culturing the host cell of claim 16 or 17 and recovering the polypeptide encoded by said polynucleotide and expressed by the host cell from the culture or the host cells.

19. A polypeptide having the amino acid sequence encoded by a polynucleotide of any one of claims 8 to 10 or obtainable by the process of claim 18.

20. A polypeptide having Rpi-blb2 activity.

21. An antibody that binds specifically to the polypeptide of claim 19 or 20.

22. An antisense nucleic acid molecule comprising the complementary sequence of the polynucleotide of any one of claims 8 to 10.

23. A method for the production of a transgenic plant, plant cell or plant tissue or a part thereof comprising the introduction of the polynucleotide of any one of claims 8 to 10 or said polynucleotide and a polynucleotide encoding a further resistance protein, or the vector of any one of claims 12 to 14 into the genome of said plant, plant tissue or plant cell or a part thereof.

24. A plant cell comprising the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14 or obtainable by the method of claim 23.

25. A transgenic plant or plant tissue or a part thereof comprising the plant cell of claim 24.

26. A method for producing a plant or a part thereof resistant to a plant pathogen of the phylum Oomyceta comprising the step:
expressing in the plant or a part thereof the polypeptide of claim 19 or 20 and a further resistance protein.

27. A method for producing a plant or a part thereof with a durable resistance to a Phytophthora sp. comprising co-expressing in the plant or a part thereof Rpi-blb and Rpi-blb2 protein or the polypeptide of claim 19 or 20.

28. The transgenic plant or plant tissue of claim 25 or produced according to claim 26 or 27, which upon the presence of the polynucleotide or the vector is resistant to a plant pathogen of the phylum Oomyceta.

29. Harvestable parts of the transgenic plant or plant tissue of claim 25 comprising the plant cell of claim 24.

30. Propagation material of the transgenic plant or plant tissue of claim 25 comprising the plant cell of claim 24.

31. Use of the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, or the polypeptide of claim 19 or 20 for producing a plant or a plant tissue, plant organ, or a plant cell or a part thereof resistant to a plant pathogen of the phylum Oomyceta.

32. A method for the identification of an compound stimulating resistance to a plant pathogen of the phylum Oomyceta comprising:

(a) contacting cells which express the polypeptide of claim 19 or 20 or its mRNA with a candidate compound under cell cultivation conditions;
(b) assaying an increase in expression of said polypeptide or said mRNA;
(c) comparing the expression level to a standard response made in the absence of said candidate compound; whereby, an increased expression over the standard indicates that the compound is stimulating resistance.

33. Use of the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, the polypeptide of claim 19 or 20 or the antibody of claim 21, for identifying and/or producing compounds activating or stimulating plant resistance to a plant pathogen of the phylum Oomyceta.

34. A diagnostic composition, comprising the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, the antibody of claim 21 or the antisense nucleic acid of claim 22 and optionally suitable means for detection.

35. A kit comprising the polynucleotide of any one of claims 8 or 12, the vector of any one of claims 12 to 14, the host cell of claim 16 or 17, the polypeptide of claim 19 or 20, the antisense nucleic acid of claim 22, the antibody of claim 21, the plant cell of claim 24, the plant or plant tissue of claim 25, the harvestable part of claim 29, or the propagation material of claim 30 and optionally a polynucleotide encoding Rpi-blb, Rpi-blb protein or an antibody against Rpi-blb.

36. A method for the production of a plant crop protectant providing the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14 or the polypeptide of claim 19 or 20 or comprising the steps of the method of claim 32; and formulating the polynucleotide of any one of claims 8 to 10, the vector of of claims 12 or 14 or the polypeptide of claim 19 or 20 or the compound identified in step (c) of claim 32 in a form applicable as agricultural composition.

37. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 36, wherein the plant pathogen is of the order Pythiales or Peronosperales.

38. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 37, wherein the plant pathogen is of the species P. infestans, Phytophthora erythroseptica, Phytophthora capsici, Phytophthora sojae, Phytophthora parasitica var. nicotianae, Bremia lactuca, Peronospera tabaci or Plasmopara viticola.

39. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 38, wherein the resistance protein is characterized by a P-loop and a NBS domain.

40. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 39, wherein the further resistance gene is a gene encoding Rpi-blb, R1, R-ber, Rpi1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11, Ph-1, Ph-2 and/or Ph-3.

41. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 40, wherein the further resistance protein is the Rpi-blb protein.

42. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 41 wherein the plant, plant cell or plant tissue is selected from the group consisting of Menyanthaceae, Solanaceae, Sclerophylacaceae, Duckeodendraceae, Goetzeaceae, Convolvulaceae, Cuscutaceae, Polemoniaceae, and Hydrophyllaceae according to the Systema Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof.

43. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one of claims 1 to 42, wherein the polynucleotide, the polypeptide, the plant cell, the host cell, the plant tissue or the plant is derived from the Solanceae family,

preferably S. bulbocastanum, potato (S. tuberosum), tomato (S. lycopersicum or Lycopersicon lycopersicum (L.) Karsten ex Farwell), petunia, tree tomato (S. betaceum), pear melon (S. muricatum) or eggplant (S. melongena).

Resistant plants and uses thereof

The present invention relates to a novel method for increasing the resitance of a plant, in particular of a Solanaceae, preferably of potato and tomato, to plant pathogens of the phylum Oomyceta comprising increasing the activity of the polypeptid of the present invention. The invention further relates to polynucleotides and vectors comprising these polynucleotdes. The invention furthermore relates to corresponding vectors, cells, transgenic plants and transgenic propagation material derived from them, methods to produce them and to their use for the production of foodstuffs, feeding stuffs, seed, pharmaceuticals or fine chemicals.

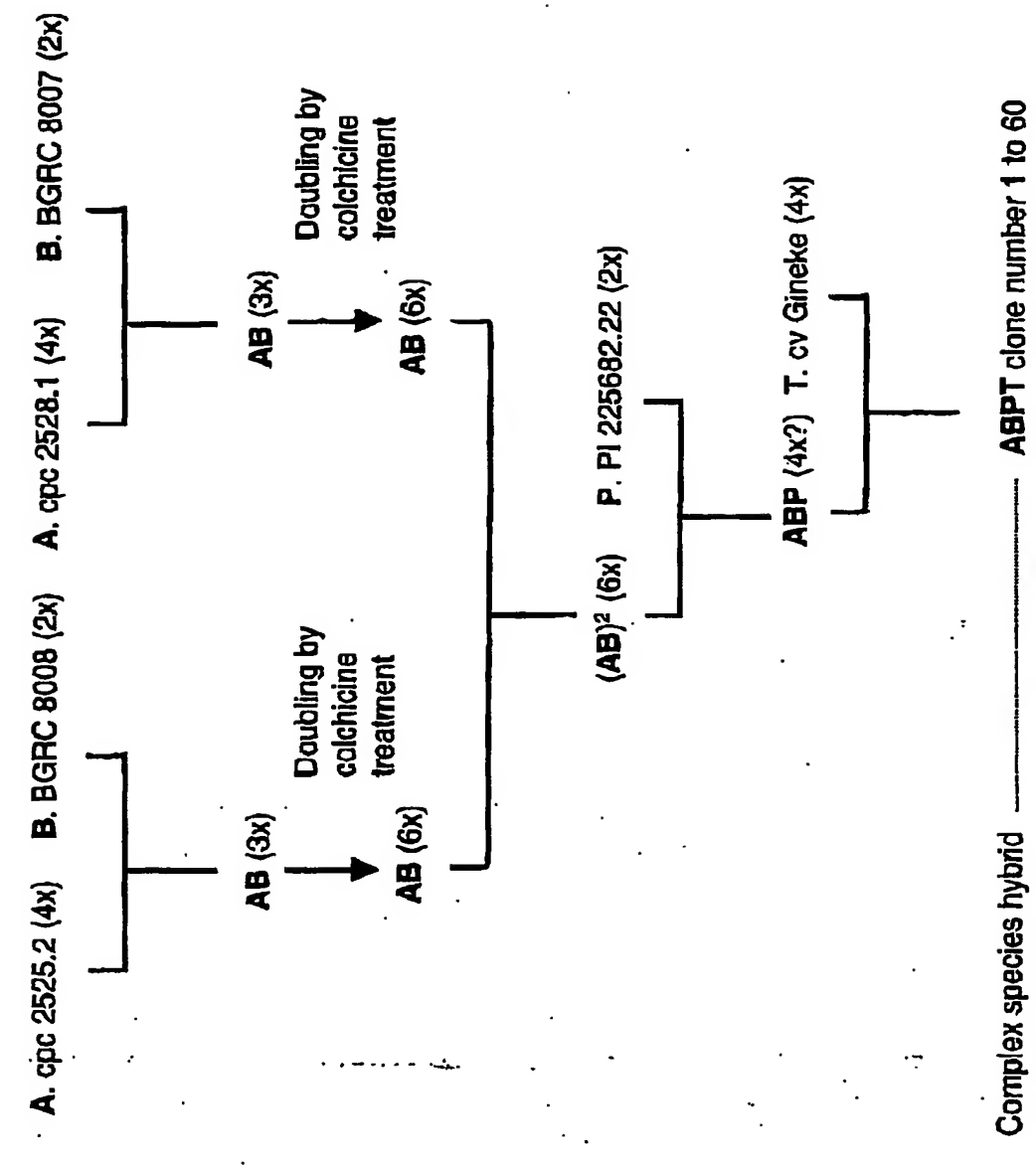

A. cpc 2525.2 (4x)
B. BGRC 8008 (2x)
AB (3x)
Doubling by colchicine treatment
AB (6x)
A. cpc 2528.1 (4x)
B. BGRC 8007 (2x)
AB (3x)
Doubling by colchicine treatment
AB (6x)
(AB)² (6x)
P. PI 225682.22 (2x)
ABP (4x?)
T. cv Gineke (4x)
Complex species hybrid
ABPT clone number 1 to 60

Figure 1A

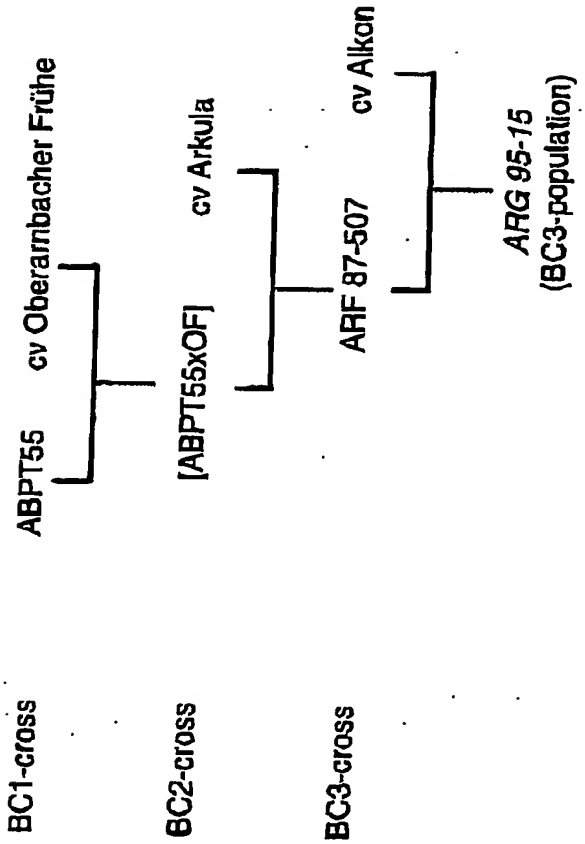

BC1-cross
ABPT55
cv Oberarnbacher Frühe
BC2-cross
[ABPT55xOF]
cv Arkula
BC3-cross
ARF 87-507
cv Alkon
ARG 95-15
(BC3-population)

Figure 1B

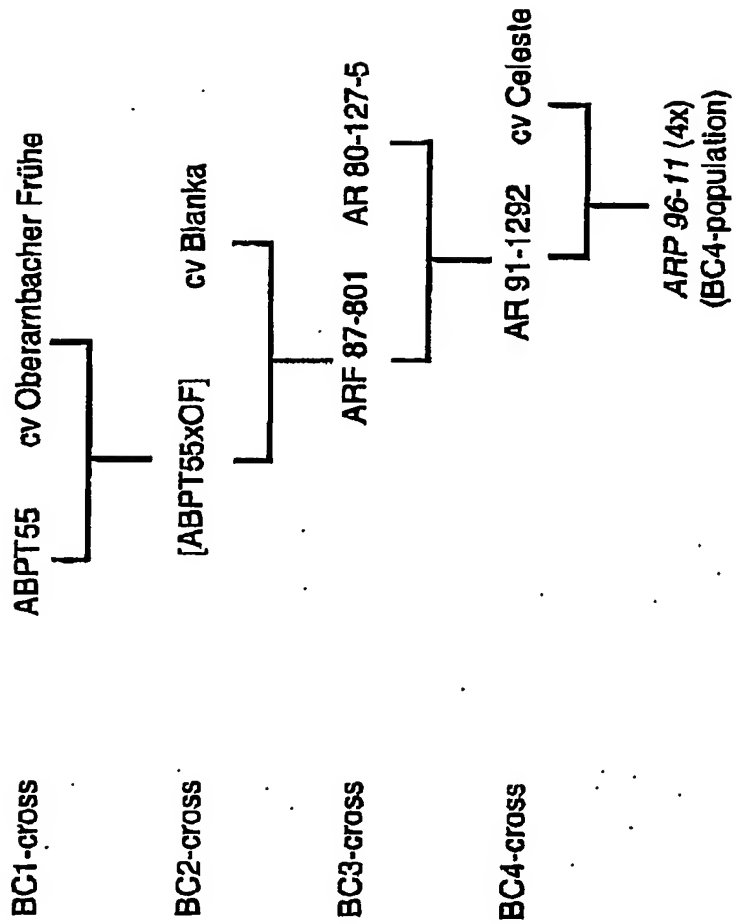

BC1-cross
ABPT55
cv Oberarnbacher Frühe
BC2-cross
[ABPT55xOF]
cv Blanka
BC3-cross
ARF 87-801
AR 80-127-5
BC4-cross
AR 91-1292
cv Celeste
ARP 96-11 (4x)
(BC4-population)

Figure 1C

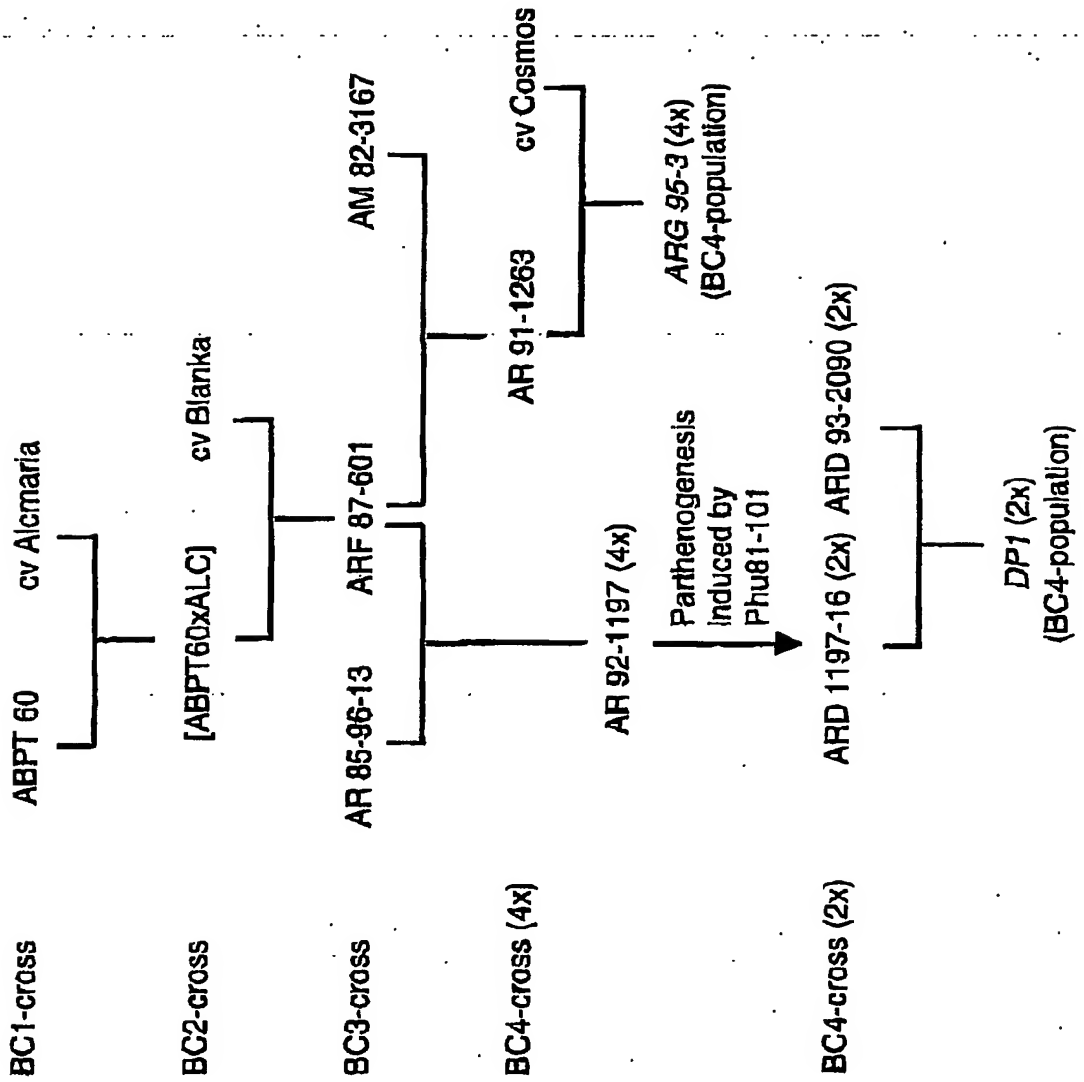

BC1-cross
ABPT 60
cv Alcmaria
BC2-cross
[ABPT60xALC]
cv Blanka
BC3-cross
AR 85-96-13
ARF 87-601
AM 82-3167
BC4-cross (4x)
AR 92-1197 (4x)
AR 91-1263
cv Cosmos
Parthenogenesis induced by Phu81-101
ARG 95-3 (4x) (BC4-population)
BC4-cross (2x)
ARD 1197-16 (2x)
ARD 93-2090 (2x)
DP1 (2x) (BC4-population)

Figure 1D

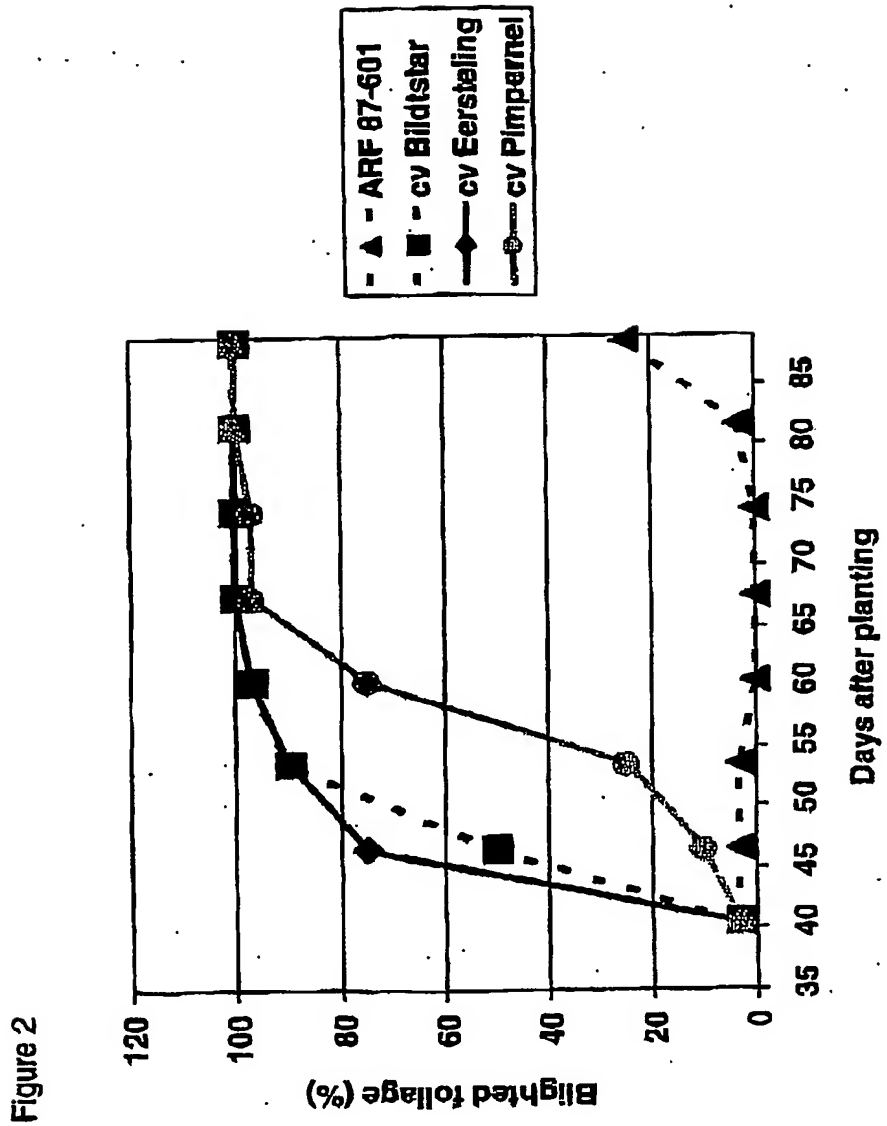

ARF 87-601
cv Bildtstar
cv Eersteling
cv Pimpernel
Blighted foliage (%)
120
100
80
60
40
20
0
35
40
45
50
55
60
65
70
75
80
85
Days after planting

Figure 2

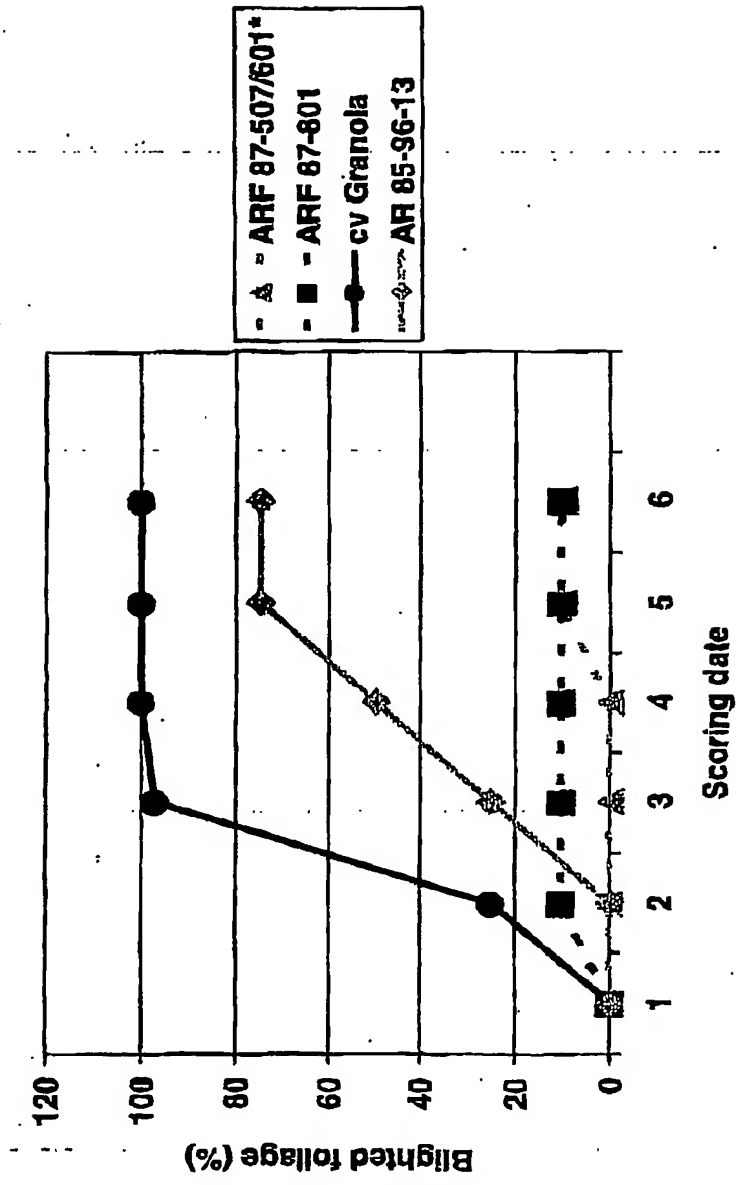
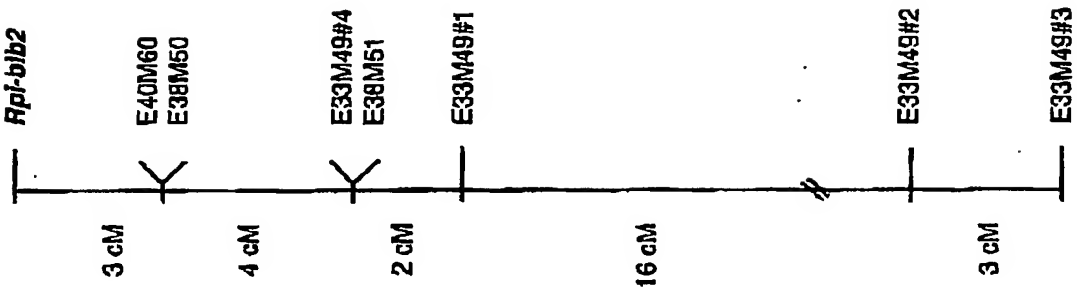


Figure 3

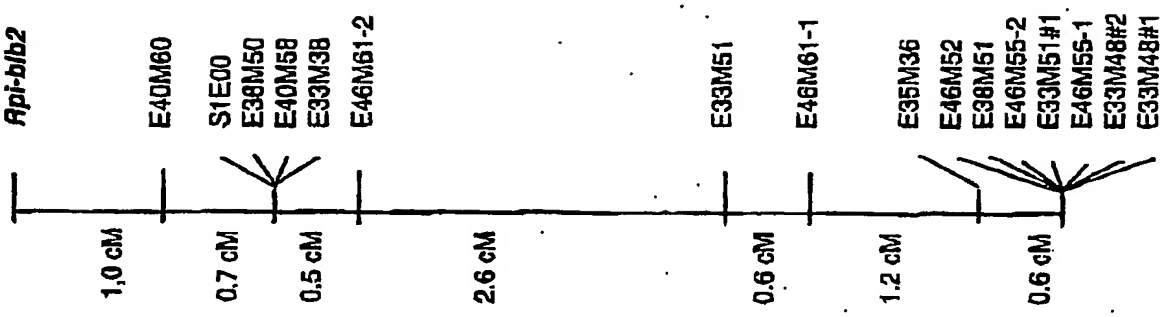
* ARF 87-507 and ARF 87-601 had identical disease progress curves

Figure 4



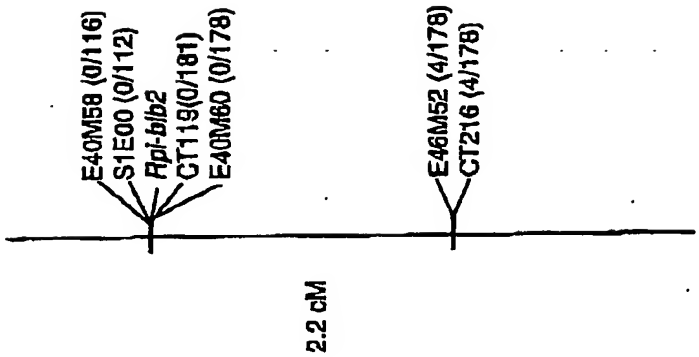
ARG 95-15

Figure 5



ARG 95-3

Figure 6



ARG 95-3

Figure 7

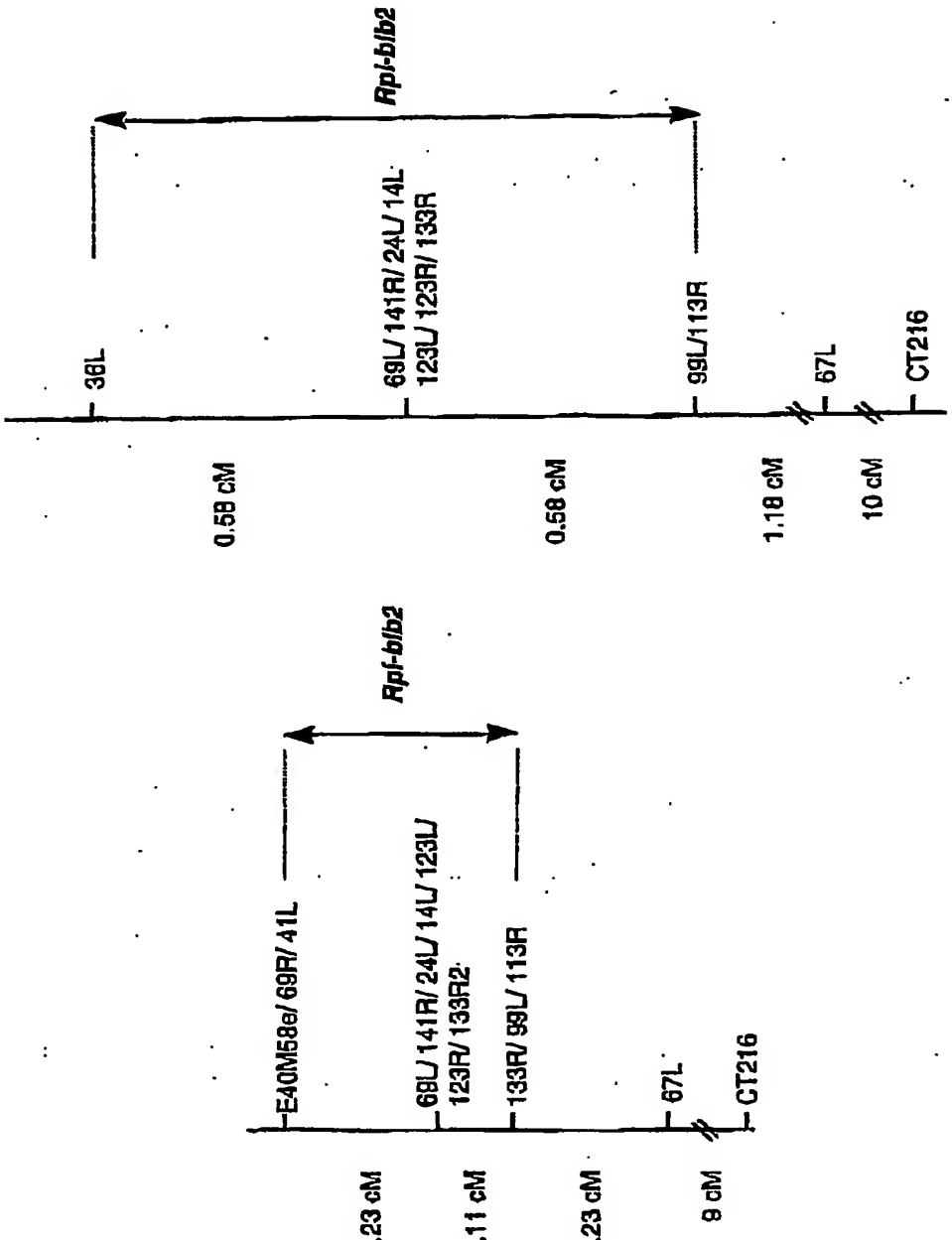

Rpi-blb2
36L
69L/141R/24L/14L
123L/123R/133R
99L/113R
67L
CT216
0.58 cM
0.58 cM
1.18 cM
10 cM
ARP 96-11
Rpi-blb2
E40M58e/69R/41L
68L/141R/24L/14L/123L
123R/133R2
133R/99L/113R
67L
CT216
0.23 cM
0.11 cM
0.23 cM
8 cM
ARG 95-3

Figure 8

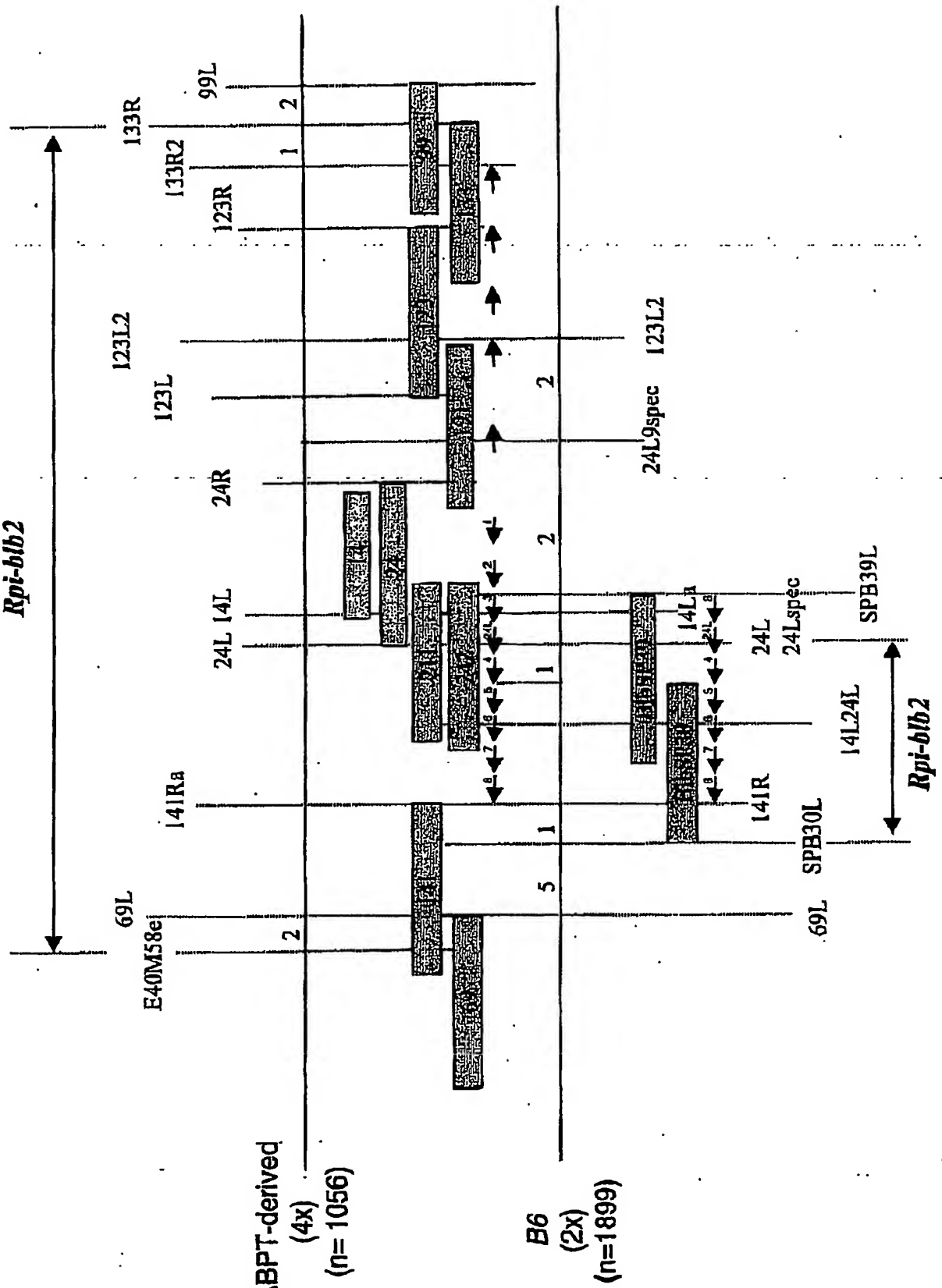

Rpi-blb2
133R
133R2
123R
123L2
123L
24R
24L
14L
141Ra
69L
E40M58e
99L
123L2
24L9spec
14L
24L
24Lspec
SPB39L
14L24L
141R
SPB30L
69L
Rpi-blb2
ABPT-derived
(4x)
(n= 1056)
B6
(2x)
(n=1899)

Figure 9

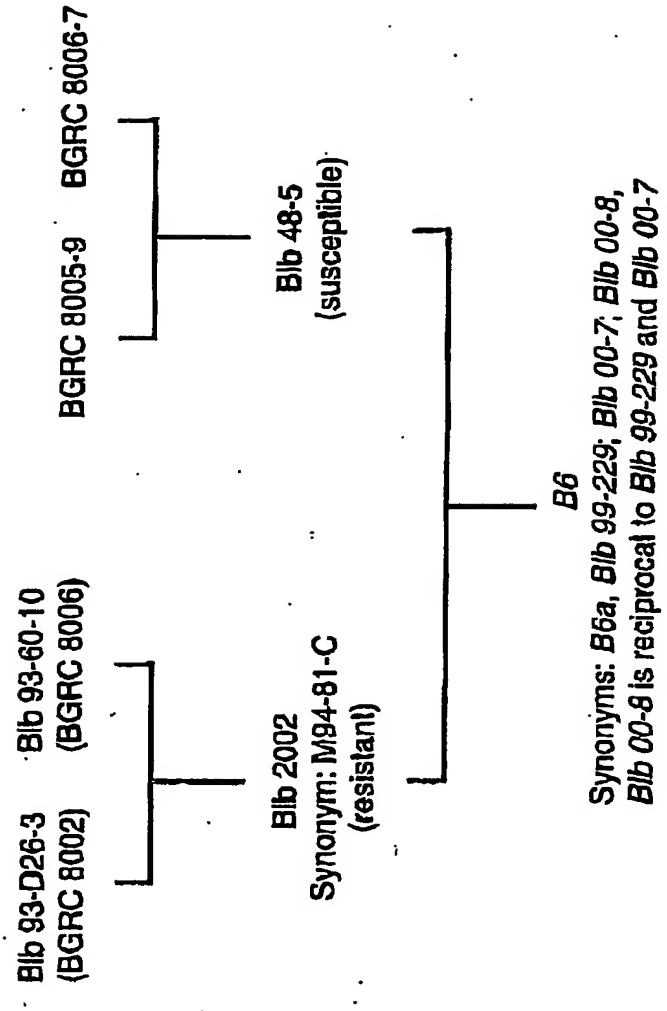

Bib 93-D26-3
(BGRC 8002)
Bib 93-60-10
(BGRC 8006)
Bib 2002
Synonym: M94-81-C
(resistant)
BGRC 8005-9
BGRC 8006-7
Bib 48-5
(susceptible)
B6
Synonyms: B6a, Bib 99-229; Bib 00-7; Bib 00-8,
Bib 00-8 is reciprocal to Bib 99-229 and Bib 00-7

Figure 10

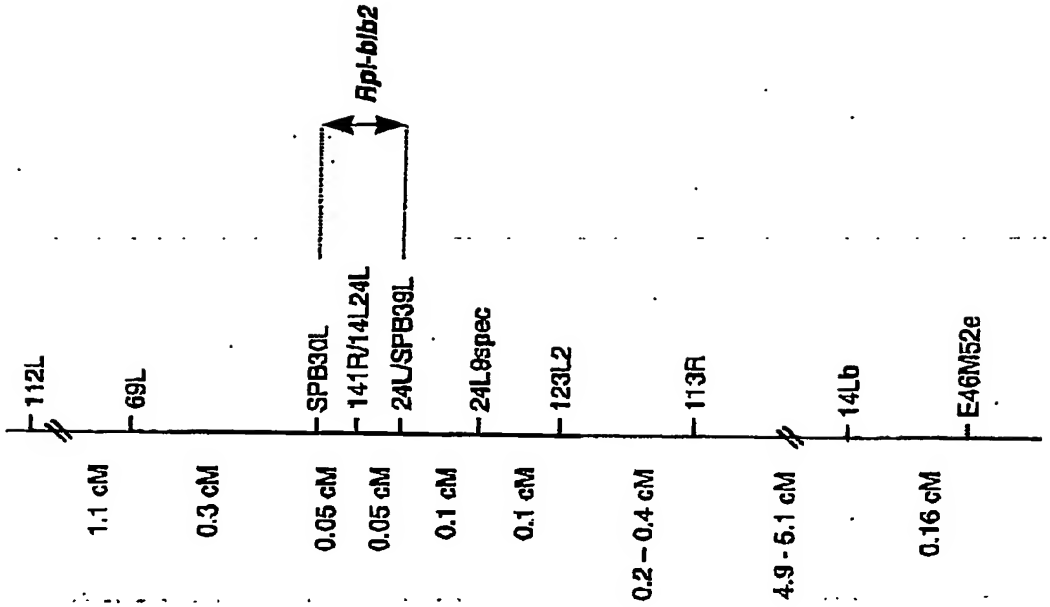

Rpi-blb2
112L
69L
SPB30L
141R/14L24L
24USPB39L
24L8spec
123L2
113R
14Lb
E46M52e
1.1 cM
0.3 cM
0.05 cM
0.05 cM
0.1 cM
0.1 cM
0.2 – 0.4 cM
4.9 - 5.1 cM
0.16 cM
B6

Figure 11

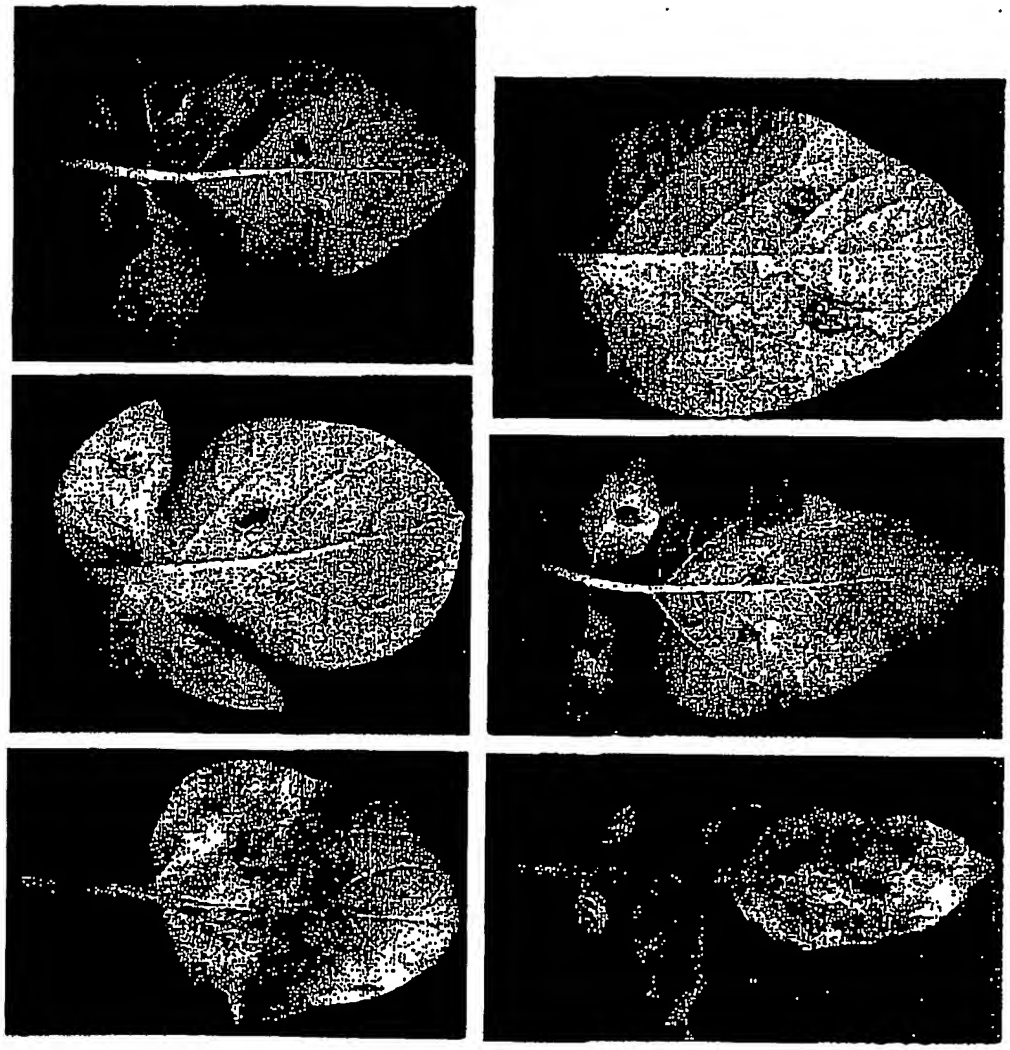


Figure 12

Agrico B.V. **20030596** **PF 54801**

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Figure 13A

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CTGAAATTGAAGCTGACATTTATTTGTACATATGTCCAGCTTTCTTATTC 200
CGATTTGGAGAAGTTTGAAGATATAATGACTAGAAAAAGACAAGAGGTTG 250
AGAATCTGCTTCAACCAATTTTGGATGATGATGGCAAAGACGTCGGGTGT 300
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3

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Figure 13B

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Figure 13C

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10

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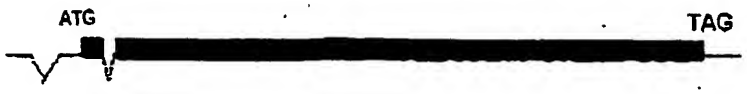

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Figure 14

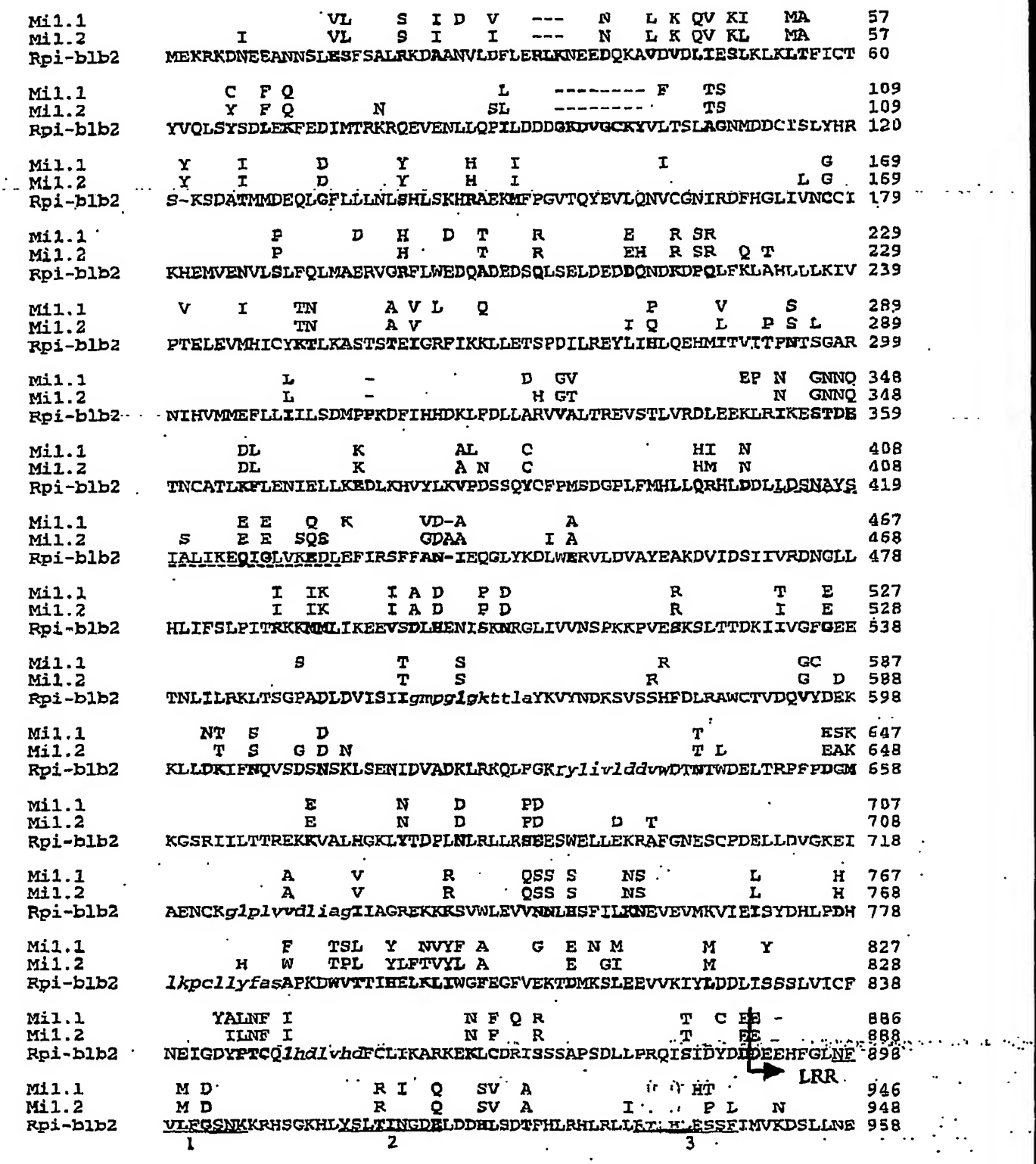

Figure 15

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Figure 15

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1               5                  10                  15

ttt tct gct ctt cgc aag gat gct gcc aat gtt ctg gat ttc cta gag      96
Phe Ser Ala Leu Arg Lys Asp Ala Ala Asn Val Leu Asp Phe Leu Glu
            20                  25                  30

aga tta aag aat gaa gaa gat caa aag gct gtt gat gtg gat ctg att     144
Arg Leu Lys Asn Glu Glu Asp Gln Lys Ala Val Asp Val Asp Leu Ile
        35                  40                  45

gaa agc ctg aaa ttg aag ctg aca ttt att tgt aca tat gtc cag ctt     192
Glu Ser Leu Lys Leu Lys Leu Thr Phe Ile Cys Thr Tyr Val Gln Leu
    50                  55                  60

tct tat tcc gat ttg gag aag ttt gaa gat ata atg act aga aaa aga     240
Ser Tyr Ser Asp Leu Glu Lys Phe Glu Asp Ile Met Thr Arg Lys Arg
65                  70                  75                  80

caa gag gtt gag aat ctg ctt caa cca att ttg gat gat gat ggc aaa     288
Gln Glu Val Glu Asn Leu Leu Gln Pro Ile Leu Asp Asp Asp Gly Lys
                85                  90                  95

gac gtc ggg tgt aaa tat gtc ctt act agc ctc gcc ggt aat atg gat     336
Asp Val Gly Cys Lys Tyr Val Leu Thr Ser Leu Ala Gly Asn Met Asp
            100                 105                 110

gac tgt ata agc ttg tat cat cgt tct aaa tca gat gcc acc atg atg     384
Asp Cys Ile Ser Leu Tyr His Arg Ser Lys Ser Asp Ala Thr Met Met
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gac tgt ata agc ttg tat cat cgt tct aaa tca gat gcc acc atg atg      384
Asp Cys Ile Ser Leu Tyr His Arg Ser Lys Ser Asp Ala Thr Met Met
        115                 120                 125

gat gag caa ttg ggc ttc ctc ctc ttg aat ctc tct cat cta tcc aag      432
Asp Glu Gln Leu Gly Phe Leu Leu Leu Asn Leu Ser His Leu Ser Lys
    130                 135                 140

cat cgt gct gaa aag atg ttt cct gga gtg act caa tat gag gtt ctt      480
His Arg Ala Glu Lys Met Phe Pro Gly Val Thr Gln Tyr Glu Val Leu
145                 150                 155                 160

cag aat gta tgt ggc aac ata aga gat ttc cat gga ttg ata gtg aat      528
Gln Asn Val Cys Gly Asn Ile Arg Asp Phe His Gly Leu Ile Val Asn
                165                 170                 175

tgt tgc att aag cat gag atg gtt gag aat gtc tta tct ctg ttt caa      576
Cys Cys Ile Lys His Glu Met Val Glu Asn Val Leu Ser Leu Phe Gln
            180                 185                 190

ctg atg gct gag aga gta gga cgc ttc ctt tgg gag gat cag gct gat      624
Leu Met Ala Glu Arg Val Gly Arg Phe Leu Trp Glu Asp Gln Ala Asp
        195                 200                 205

gaa gac tct caa ctc tcc gag cta gat gag gat gat cag aat gat aaa      672
Glu Asp Ser Gln Leu Ser Glu Leu Asp Glu Asp Asp Gln Asn Asp Lys
    210                 215                 220

gac cct caa ctc ttc aag cta gca cat cta ctc ttg aag att gtt cca      720
Asp Pro Gln Leu Phe Lys Leu Ala His Leu Leu Leu Lys Ile Val Pro
225                 230                 235                 240

act gaa ttg gag gtt atg cac ata tgt tat aaa act ttg aaa gct tca      768
Thr Glu Leu Glu Val Met His Ile Cys Tyr Lys Thr Leu Lys Ala Ser
                245                 250                 255

act tca aca gaa att gga cgc ttc att aag aag ctc ctg gaa acc tct      816
Thr Ser Thr Glu Ile Gly Arg Phe Ile Lys Lys Leu Leu Glu Thr Ser
            260                 265                 270

ccg gac att ctc aga gaa tat ctg att cat cta caa gag cat atg ata      864
Pro Asp Ile Leu Arg Glu Tyr Leu Ile His Leu Gln Glu His Met Ile
        275                 280                 285

act gtt att acc cct aac act tca ggg gct cga aac att cat gtc atg      912
Thr Val Ile Thr Pro Asn Thr Ser Gly Ala Arg Asn Ile His Val Met
    290                 295                 300

atg gaa ttc cta ttg att att ctt tct gat atg ccg ccc aag gac ttt      960
Met Glu Phe Leu Leu Ile Ile Leu Ser Asp Met Pro Pro Lys Asp Phe
305                 310                 315                 320

att cat cat gac aaa ctt ttt gat ctc ttg gct cgt gtt gta gca ctt     1008
Ile His His Asp Lys Leu Phe Asp Leu Leu Ala Arg Val Val Ala Leu
                325                 330                 335

acc agg gag gta tca act ctt gta cgc gac ttg gaa gag aaa tta agg     1056
Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu Glu Glu Lys Leu Arg
            340                 345                 350

att aaa gag agt act gac gaa aca aat tgt gca acc cta aag ttt ctg     1104
Ile Lys Glu Ser Thr Asp Glu Thr Asn Cys Ala Thr Leu Lys Phe Leu
        355                 360                 365

gaa aat att gaa ctc ctt aag gaa gat ctc aaa cat gtt tat ctg aaa     1152
Glu Asn Ile Glu Leu Leu Lys Glu Asp Leu Lys His Val Tyr Leu Lys
    370                 375                 380

gtc ccg gat tca tct caa tat tgc ttc ccc atg agt gat gga cct ctc     1200
Val Pro Asp Ser Ser Gln Tyr Cys Phe Pro Met Ser Asp Gly Pro Leu
385                 390                 395                 400

ttc atg cat ctg cta cag aga cac tta gat gat ttg ctg gat tcc aat     1248
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Phe Met His Leu Leu Gln Arg His Leu Asp Asp Leu Leu Asp Ser Asn
405 410 415

gct tat tca att gct ttg ata aag gaa caa att ggg ctg gtg aaa gaa 1296
Ala Tyr Ser Ile Ala Leu Ile Lys Glu Gln Ile Gly Leu Val Lys Glu
420 425 430

gac ttg gaa ttc ata aga tct ttt ttc gcg aat att gag caa gga ttg 1344
Asp Leu Glu Phe Ile Arg Ser Phe Phe Ala Asn Ile Glu Gln Gly Leu
435 440 445

tat aaa gat ctc tgg gaa cgt gtt cta gat gtg gca tat gag gca aaa 1392
Tyr Lys Asp Leu Trp Glu Arg Val Leu Asp Val Ala Tyr Glu Ala Lys
450 455 460

gat gtc ata gat tca att att gtt cga gat aat ggt ctc tta cat ctt 1440
Asp Val Ile Asp Ser Ile Ile Val Arg Asp Asn Gly Leu Leu His Leu
465 470 475 480

att ttc tca ctt ccc att acc aga aag aag atg atg ctt atc aaa gaa 1488
Ile Phe Ser Leu Pro Ile Thr Arg Lys Lys Met Met Leu Ile Lys Glu
485 490 495

gag gtc tct gat tta cat gag aac att tcc aag aac aga ggt ctc atc 1536
Glu Val Ser Asp Leu His Glu Asn Ile Ser Lys Asn Arg Gly Leu Ile
500 505 510

gtt gtg aac tct ccc aag aaa cca gtt gag agc aag tca ttg aca act 1584
Val Val Asn Ser Pro Lys Lys Pro Val Glu Ser Lys Ser Leu Thr Thr
515 520 525

gat aaa ata att gta ggt ttt ggt gag gag aca aac ttg ata ctt aga 1632
Asp Lys Ile Ile Val Gly Phe Gly Glu Glu Thr Asn Leu Ile Leu Arg
530 535 540

aag ctc acc agt gga ccg gca gat cta gat gtc att tcg atc att ggt 1680
Lys Leu Thr Ser Gly Pro Ala Asp Leu Asp Val Ile Ser Ile Ile Gly
545 550 555 560

atg ccg ggt tta ggt aaa act act ttg gcg tac aaa gta tac aat gat 1728
Met Pro Gly Leu Gly Lys Thr Thr Leu Ala Tyr Lys Val Tyr Asn Asp
565 570 575

aaa tca gtt tct agc cat ttc gac ctt cgt gca tgg tgc acg gtc gac 1776
Lys Ser Val Ser Ser His Phe Asp Leu Arg Ala Trp Cys Thr Val Asp
580 585 590

caa gta tat gac gag aag aag ttg ttg gat aaa att ttc aat caa gtt 1824
Gln Val Tyr Asp Glu Lys Lys Leu Leu Asp Lys Ile Phe Asn Gln Val
595 600 605

agt gac tca aat tca aaa ttg agt gag aat att gat gtt gct gat aaa 1872
Ser Asp Ser Asn Ser Lys Leu Ser Glu Asn Ile Asp Val Ala Asp Lys
610 615 620

cta cgg aaa caa ttg ttt gga aag agg tat ctt att gtc tta gat gac 1920
Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr Leu Ile Val Leu Asp Asp
625 630 635 640

gtg tgg gat act aat aca tgg gat gag cta aca aga cct ttt cct gat 1968
Val Trp Asp Thr Asn Thr Trp Asp Glu Leu Thr Arg Pro Phe Pro Asp
645 650 655

ggt atg aaa gga agt aga att att ttg aca act cga gaa aag aaa gtt 2016
Gly Met Lys Gly Ser Arg Ile Ile Leu Thr Thr Arg Glu Lys Lys Val
660 665 670

gct ttg cat gga aag ctc tac act gat cct ctt aac ctt cga ttg cta 2064
Ala Leu His Gly Lys Leu Tyr Thr Asp Pro Leu Asn Leu Arg Leu Leu
675 680 685

aga tca gaa gaa agt tgg gag tta tta gag aaa agg gca ttt gga aac 2112
Arg Ser Glu Glu Ser Trp Glu Leu Leu Glu Lys Arg Ala Phe Gly Asn

690 695 700

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gag agt tgc cct gat gaa cta ttg gat gtt ggt aaa gaa ata gcc gaa      2160
Glu Ser Cys Pro Asp Glu Leu Leu Asp Val Gly Lys Glu Ile Ala Glu
705                 710                 715                 720

aat tgt aaa ggg ctt cct ttg gtg gtg gat ctg att gct gga atc att      2208
Asn Cys Lys Gly Leu Pro Leu Val Val Asp Leu Ile Ala Gly Ile Ile
                725                 730                 735

gct ggg agg gaa aag aaa aag agt gtg tgg ctt gaa gtt gta aat aat      2256
Ala Gly Arg Glu Lys Lys Lys Ser Val Trp Leu Glu Val Val Asn Asn
            740                 745                 750

ttg cat tcc ttt att ttg aag aat gaa gtg gaa gtg atg aaa gtt ata      2304
Leu His Ser Phe Ile Leu Lys Asn Glu Val Glu Val Met Lys Val Ile
        755                 760                 765

gaa ata agt tat gac cac tta cct gat cac ctg aag cca tgc ttg ctg      2352
Glu Ile Ser Tyr Asp His Leu Pro Asp His Leu Lys Pro Cys Leu Leu
    770                 775                 780

tac ttt gca agt gcg ccg aag gac tgg gta acg aca atc cat gag ttg      2400
Tyr Phe Ala Ser Ala Pro Lys Asp Trp Val Thr Thr Ile His Glu Leu
785                 790                 795                 800

aaa ctt att tgg ggt ttt gaa gga ttt gtg gaa aag aca gat atg aag      2448
Lys Leu Ile Trp Gly Phe Glu Gly Phe Val Glu Lys Thr Asp Met Lys
                805                 810                 815

agt ctg gaa gaa gtg gtg aaa att tat ttg gat gat tta att tcc agt      2496
Ser Leu Glu Glu Val Val Lys Ile Tyr Leu Asp Asp Leu Ile Ser Ser
            820                 825                 830

agc ttg gta att tgt ttc aat gag ata ggt gat tac cct act tgc caa      2544
Ser Leu Val Ile Cys Phe Asn Glu Ile Gly Asp Tyr Pro Thr Cys Gln
        835                 840                 845

ctt cat gat ctt gtg cat gac ttt tgt ttg ata aaa gca aga aag gaa      2592
Leu His Asp Leu Val His Asp Phe Cys Leu Ile Lys Ala Arg Lys Glu
    850                 855                 860

aag ttg tgt gat cgg ata agt tca agt gct cca tca gat ttg ttg cca      2640
Lys Leu Cys Asp Arg Ile Ser Ser Ser Ala Pro Ser Asp Leu Leu Pro
865                 870                 875                 880

cgt caa att agc att gat tat gat gat gat gaa gag cac ttt ggg ctt      2688
Arg Gln Ile Ser Ile Asp Tyr Asp Asp Asp Glu Glu His Phe Gly Leu
                885                 890                 895

aat ttt gtc ctg ttc ggt tca aat aag aaa agg cat tcc ggt aaa cac      2736
Asn Phe Val Leu Phe Gly Ser Asn Lys Lys Arg His Ser Gly Lys His
            900                 905                 910

ctc tat tct ttg acc ata aat gga gat gag ctg gac gac cat ctt tct      2784
Leu Tyr Ser Leu Thr Ile Asn Gly Asp Glu Leu Asp Asp His Leu Ser
        915                 920                 925

gat aca ttt cat cta aga cac ttg agg ctt ctt aga acc ttg cac ctg      2832
Asp Thr Phe His Leu Arg His Leu Arg Leu Leu Arg Thr Leu His Leu
    930                 935                 940

gaa tcc tct ttt atc atg gtt aaa gat tct ttg ctg aat gaa ata tgc      2880
Glu Ser Ser Phe Ile Met Val Lys Asp Ser Leu Leu Asn Glu Ile Cys
945                 950                 955                 960

atg ttg aat cat ttg agg tac tta agc att ggg aca gaa gtt aaa tct      2928
Met Leu Asn His Leu Arg Tyr Leu Ser Ile Gly Thr Glu Val Lys Ser
                965                 970                 975

ctg cct ttg tct ttc tca aac ctc tgg aat cta gaa atc ttg ttt gtg      2976
Leu Pro Leu Ser Phe Ser Asn Leu Trp Asn Leu Glu Ile Leu Phe Val
            980                 985                 990
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gat aac aaa gaa tca acc ttg ata  cta tta ccg aga att  tgg gat ctt   3024
Asp Asn Lys Glu Ser Thr Leu Ile  Leu Leu Pro Arg Ile  Trp Asp Leu
        995                 1000                1005

gta aag  ttg caa gtg ctg ttc  acg act gct tgt tct  ttc ttt gat      3069
Val Lys  Leu Gln Val Leu Phe  Thr Thr Ala Cys Ser  Phe Phe Asp
    1010                1015                1020

atg gat  gca gat gaa tca ata  ctg ata gca gag gac  aca aag tta      3114
Met Asp  Ala Asp Glu Ser Ile  Leu Ile Ala Glu Asp  Thr Lys Leu
    1025                1030                1035

gag aac  ttg aca gca tta ggg  gaa ctc gtg ctt tcc  tat tgg aaa      3159
Glu Asn  Leu Thr Ala Leu Gly  Glu Leu Val Leu Ser  Tyr Trp Lys
    1040                1045                1050

gat aca  gag gat att ttc aaa  agg ctt ccc aat ctt  caa gtg ctt      3204
Asp Thr  Glu Asp Ile Phe Lys  Arg Leu Pro Asn Leu  Gln Val Leu
    1055                1060                1065

cat ttc  aaa ctc aag gag tca  tgg gat tat tca aca  gag caa tat      3249
His Phe  Lys Leu Lys Glu Ser  Trp Asp Tyr Ser Thr  Glu Gln Tyr
    1070                1075                1080

tgg ttc  ccg aaa ttg gat ttc  cta act gaa cta gaa  aaa ctc act      3294
Trp Phe  Pro Lys Leu Asp Phe  Leu Thr Glu Leu Glu  Lys Leu Thr
    1085                1090                1095

gta gat  ttt gaa aga tca aac  aca aat gac agt ggg  tcc tct gca      3339
Val Asp  Phe Glu Arg Ser Asn  Thr Asn Asp Ser Gly  Ser Ser Ala
    1100                1105                1110

gcc ata  aat cgg cca tgg gat  ttt cac ttt cct tcg  agt ttg aaa      3384
Ala Ile  Asn Arg Pro Trp Asp  Phe His Phe Pro Ser  Ser Leu Lys
    1115                1120                1125

aga ttg  caa ttg cat gaa ttt  cct ctg aca tcc gat  tca cta tca      3429
Arg Leu  Gln Leu His Glu Phe  Pro Leu Thr Ser Asp  Ser Leu Ser
    1130                1135                1140

aca ata  gcg aga ctg ctg aac  ctt gaa gag ttg tac  ctt tat cgt      3474
Thr Ile  Ala Arg Leu Leu Asn  Leu Glu Glu Leu Tyr  Leu Tyr Arg
    1145                1150                1155

aca atc  atc cat ggg gaa gaa  tgg aac atg gga gaa  gaa gac acc      3519
Thr Ile  Ile His Gly Glu Glu  Trp Asn Met Gly Glu  Glu Asp Thr
    1160                1165                1170

ttt gag  aat ctc aaa tgt ttg  atg ttg agt caa gtg  att ctt tcc      3564
Phe Glu  Asn Leu Lys Cys Leu  Met Leu Ser Gln Val  Ile Leu Ser
    1175                1180                1185

aag tgg  gag gtt gga gag gaa  tct ttt ccc acg ctt  gag aaa tta      3609
Lys Trp  Glu Val Gly Glu Glu  Ser Phe Pro Thr Leu  Glu Lys Leu
    1190                1195                1200

gaa ctg  tcg gac tgt cat aat  ctt gag gag att ccg  tct agt ttt      3654
Glu Leu  Ser Asp Cys His Asn  Leu Glu Glu Ile Pro  Ser Ser Phe
    1205                1210                1215

ggg gat  att tat tcc ttg aaa  att atc gaa ctt gta  agg agc cct      3699
Gly Asp  Ile Tyr Ser Leu Lys  Ile Ile Glu Leu Val  Arg Ser Pro
    1220                1225                1230

caa ctt  gaa aat tcc gct ctc  aag att aag gaa tat  gct gaa gat      3744
Gln Leu  Glu Asn Ser Ala Leu  Lys Ile Lys Glu Tyr  Ala Glu Asp
    1235                1240                1245

atg agg  gga ggg gac gag ctt  cag atc ctt ggc cag  aag gat atc      3789
Met Arg  Gly Gly Asp Glu Leu  Gln Ile Leu Gly Gln  Lys Asp Ile
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ccg tta ttt aag tag 3804
Pro Leu Phe Lys
1265

<210> 2

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<212> PRT

<213> Solanum bulbocastanum

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Arg Leu Lys Asn Glu Glu Asp Gln Lys Ala Val Asp Val Asp Leu Ile
35 40 45

Glu Ser Leu Lys Leu Lys Leu Thr Phe Ile Cys Thr Tyr Val Gln Leu
50 55 60

Ser Tyr Ser Asp Leu Glu Lys Phe Glu Asp Ile Met Thr Arg Lys Arg
65 70 75 80

Gln Glu Val Glu Asn Leu Leu Gln Pro Ile Leu Asp Asp Asp Gly Lys
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Asp Val Gly Cys Lys Tyr Val Leu Thr Ser Leu Ala Gly Asn Met Asp
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Asp Cys Ile Ser Leu Tyr His Arg Ser Lys Ser Asp Ala Thr Met Met
115 120 125

Asp Glu Gln Leu Gly Phe Leu Leu Leu Asn Leu Ser His Leu Ser Lys
130 135 140

His Arg Ala Glu Lys Met Phe Pro Gly Val Thr Gln Tyr Glu Val Leu
145 150 155 160

Gln Asn Val Cys Gly Asn Ile Arg Asp Phe His Gly Leu Ile Val Asn
165 170 175

Cys Cys Ile Lys His Glu Met Val Glu Asn Val Leu Ser Leu Phe Gln
180 185 190

Leu Met Ala Glu Arg Val Gly Arg Phe Leu Trp Glu Asp Gln Ala Asp
195 200 205

Glu Asp Ser Gln Leu Ser Glu Leu Asp Glu Asp Asp Gln Asn Asp Lys
210 215 220

Asp Pro Gln Leu Phe Lys Leu Ala His Leu Leu Leu Lys Ile Val Pro
225 230 235 240

Thr Glu Leu Glu Val Met His Ile Cys Tyr Lys Thr Leu Lys Ala Ser
245 250 255

Thr Ser Thr Glu Ile Gly Arg Phe Ile Lys Lys Leu Leu Glu Thr Ser
260 265 270

Pro Asp Ile Leu Arg Glu Tyr Leu Ile His Leu Gln Glu His Met Ile
275 280 285

Thr Val Ile Thr Pro Asn Thr Ser Gly Ala Arg Asn Ile His Val Met
290 295 300

Met Glu Phe Leu Leu Ile Ile Leu Ser Asp Met Pro Pro Lys Asp Phe
305 310 315 320

Ile His His Asp Lys Leu Phe Asp Leu Leu Ala Arg Val Val Ala Leu
325 330 335

Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu Glu Glu Lys Leu Arg
340 345 350

Ile Lys Glu Ser Thr Asp Glu Thr Asn Cys Ala Thr Leu Lys Phe Leu
355 360 365

Glu Asn Ile Glu Leu Leu Lys Glu Asp Leu Lys His Val Tyr Leu Lys
370 375 380

Val Pro Asp Ser Ser Gln Tyr Cys Phe Pro Met Ser Asp Gly Pro Leu
385 390 395 400

Phe Met His Leu Leu Gln Arg His Leu Asp Asp Leu Leu Asp Ser Asn
405 410 415

Ala Tyr Ser Ile Ala Leu Ile Lys Glu Gln Ile Gly Leu Val Lys Glu
420 425 430

Asp Leu Glu Phe Ile Arg Ser Phe Phe Ala Asn Ile Glu Gln Gly Leu
435 440 445

Tyr Lys Asp Leu Trp Glu Arg Val Leu Asp Val Ala Tyr Glu Ala Lys
450 455 460

Asp Val Ile Asp Ser Ile Ile Val Arg Asp Asn Gly Leu Leu His Leu
465 470 475 480

Ile Phe Ser Leu Pro Ile Thr Arg Lys Lys Met Met Leu Ile Lys Glu
485 490 495

Glu Val Ser Asp Leu His Glu Asn Ile Ser Lys Asn Arg Gly Leu Ile
500 505 510

Val Val Asn Ser Pro Lys Lys Pro Val Glu Ser Lys Ser Leu Thr Thr

515 520 525

Asp Lys Ile Ile Val Gly Phe Gly Glu Glu Thr Asn Leu Ile Leu Arg
530 535 540

Lys Leu Thr Ser Gly Pro Ala Asp Leu Asp Val Ile Ser Ile Ile Gly
545 550 555 560

Met Pro Gly Leu Gly Lys Thr Thr Leu Ala Tyr Lys Val Tyr Asn Asp
565 570 575

Lys Ser Val Ser Ser His Phe Asp Leu Arg Ala Trp Cys Thr Val Asp
580 585 590

Gln Val Tyr Asp Glu Lys Lys Leu Leu Asp Lys Ile Phe Asn Gln Val
595 600 605

Ser Asp Ser Asn Ser Lys Leu Ser Glu Asn Ile Asp Val Ala Asp Lys
610 615 620

Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr Leu Ile Val Leu Asp Asp
625 630 635 640

Val Trp Asp Thr Asn Thr Trp Asp Glu Leu Thr Arg Pro Phe Pro Asp
645 650 655

Gly Met Lys Gly Ser Arg Ile Ile Leu Thr Thr Arg Glu Lys Lys Val
660 665 670

Ala Leu His Gly Lys Leu Tyr Thr Asp Pro Leu Asn Leu Arg Leu Leu
675 680 685

Arg Ser Glu Glu Ser Trp Glu Leu Leu Glu Lys Arg Ala Phe Gly Asn
690 695 700

Glu Ser Cys Pro Asp Glu Leu Leu Asp Val Gly Lys Glu Ile Ala Glu
705 710 715 720

Asn Cys Lys Gly Leu Pro Leu Val Val Asp Leu Ile Ala Gly Ile Ile
725 730 735

Ala Gly Arg Glu Lys Lys Lys Ser Val Trp Leu Glu Val Val Asn Asn
740 745 750

Leu His Ser Phe Ile Leu Lys Asn Glu Val Glu Val Met Lys Val Ile
755 760 765

Glu Ile Ser Tyr Asp His Leu Pro Asp His Leu Lys Pro Cys Leu Leu
770 775 780

Tyr Phe Ala Ser Ala Pro Lys Asp Trp Val Thr Thr Ile His Glu Leu
785 790 795 800

Lys Leu Ile Trp Gly Phe Glu Gly Phe Val Glu Lys Thr Asp Met Lys
805 810 815

Ser Leu Glu Glu Val Val Lys Ile Tyr Leu Asp Asp Leu Ile Ser Ser
820 825 830

Ser Leu Val Ile Cys Phe Asn Glu Ile Gly Asp Tyr Pro Thr Cys Gln
835 840 845

Leu His Asp Leu Val His Asp Phe Cys Leu Ile Lys Ala Arg Lys Glu
850 855 860

Lys Leu Cys Asp Arg Ile Ser Ser Ser Ala Pro Ser Asp Leu Leu Pro
865 870 875 880

Arg Gln Ile Ser Ile Asp Tyr Asp Asp Asp Glu Glu His Phe Gly Leu
885 890 895

Asn Phe Val Leu Phe Gly Ser Asn Lys Lys Arg His Ser Gly Lys His
900 905 910

Leu Tyr Ser Leu Thr Ile Asn Gly Asp Glu Leu Asp Asp His Leu Ser
915 920 925

Asp Thr Phe His Leu Arg His Leu Arg Leu Leu Arg Thr Leu His Leu
930 935 940

Glu Ser Ser Phe Ile Met Val Lys Asp Ser Leu Leu Asn Glu Ile Cys
945 950 955 960

Met Leu Asn His Leu Arg Tyr Leu Ser Ile Gly Thr Glu Val Lys Ser
965 970 975

Leu Pro Leu Ser Phe Ser Asn Leu Trp Asn Leu Glu Ile Leu Phe Val
980 985 990

Asp Asn Lys Glu Ser Thr Leu Ile Leu Leu Pro Arg Ile Trp Asp Leu
995 1000 1005

Val Lys Leu Gln Val Leu Phe Thr Thr Ala Cys Ser Phe Phe Asp
1010 1015 1020

Met Asp Ala Asp Glu Ser Ile Leu Ile Ala Glu Asp Thr Lys Leu
1025 1030 1035

Glu Asn Leu Thr Ala Leu Gly Glu Leu Val Leu Ser Tyr Trp Lys
1040 1045 1050

Asp Thr Glu Asp Ile Phe Lys Arg Leu Pro Asn Leu Gln Val Leu
1055 1060 1065

His Phe Lys Leu Lys Glu Ser Trp Asp Tyr Ser Thr Glu Gln Tyr
1070 1075 1080

Trp Phe Pro Lys Leu Asp Phe Leu Thr Glu Leu Glu Lys Leu Thr
1085 1090 1095

Val Asp Phe Glu Arg Ser Asn Thr Asn Asp Ser Gly Ser Ser Ala
1100 1105 1110

Ala Ile Asn Arg Pro Trp Asp Phe His Phe Pro Ser Ser Leu Lys
1115 1120 1125

Arg Leu Gln Leu His Glu Phe Pro Leu Thr Ser Asp Ser Leu Ser
1130 1135 1140

Thr Ile Ala Arg Leu Leu Asn Leu Glu Glu Leu Tyr Leu Tyr Arg
1145 1150 1155

Thr Ile Ile His Gly Glu Glu Trp Asn Met Gly Glu Glu Asp Thr
1160 1165 1170

Phe Glu Asn Leu Lys Cys Leu Met Leu Ser Gln Val Ile Leu Ser
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Lys Trp Glu Val Gly Glu Glu Ser Phe Pro Thr Leu Glu Lys Leu
1190 1195 1200

Glu Leu Ser Asp Cys His Asn Leu Glu Glu Ile Pro Ser Ser Phe
1205 1210 1215

Gly Asp Ile Tyr Ser Leu Lys Ile Ile Glu Leu Val Arg Ser Pro
1220 1225 1230

Gln Leu Glu Asn Ser Ala Leu Lys Ile Lys Glu Tyr Ala Glu Asp
1235 1240 1245

Met Arg Gly Gly Asp Glu Leu Gln Ile Leu Gly Gln Lys Asp Ile
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Pro Leu Phe Lys
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<213> Solanum bulbocastanum

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<221> gene

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<223> Coding nucleic acid sequence of the Rpi-blb2 gene including the intron sequence (position 43-128).

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tttggcagga gtcatttttct gctcttcgca aggatgctgc caatgttctg gatttcctag 180

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<213> Solanum bulbocastanum

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<222> (1)..(1267)

<223> Deduced Rpi-blb2 protein sequence

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35 40 45

Glu Ser Leu Lys Leu Lys Leu Thr Phe Ile Cys Thr Tyr Val Gln Leu
50 55 60

Ser Tyr Ser Asp Leu Glu Lys Phe Glu Asp Ile Met Thr Arg Lys Arg
65 70 75 80

Gln Glu Val Glu Asn Leu Leu Gln Pro Ile Leu Asp Asp Asp Gly Lys
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Asp Val Gly Cys Lys Tyr Val Leu Thr Ser Leu Ala Gly Asn Met Asp
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Asp Cys Ile Ser Leu Tyr His Arg Ser Lys Ser Asp Ala Thr Met Met
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Asp Glu Gln Leu Gly Phe Leu Leu Leu Asn Leu Ser His Leu Ser Lys
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His Arg Ala Glu Lys Met Phe Pro Gly Val Thr Gln Tyr Glu Val Leu
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Cys Cys Ile Lys His Glu Met Val Glu Asn Val Leu Ser Leu Phe Gln
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Asp Pro Gln Leu Phe Lys Leu Ala His Leu Leu Leu Lys Ile Val Pro

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245 250 255

Thr Ser Thr Glu Ile Gly Arg Phe Ile Lys Lys Leu Leu Glu Thr Ser
260 265 270

Pro Asp Ile Leu Arg Glu Tyr Leu Ile His Leu Gln Glu His Met Ile
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Thr Val Ile Thr Pro Asn Thr Ser Gly Ala Arg Asn Ile His Val Met
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Ile His His Asp Lys Leu Phe Asp Leu Leu Ala Arg Val Val Ala Leu
325 330 335

Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu Glu Glu Lys Leu Arg
340 345 350

Ile Lys Glu Ser Thr Asp Glu Thr Asn Cys Ala Thr Leu Lys Phe Leu
355 360 365

Glu Asn Ile Glu Leu Leu Lys Glu Asp Leu Lys His Val Tyr Leu Lys
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Val Pro Asp Ser Ser Gln Tyr Cys Phe Pro Met Ser Asp Gly Pro Leu
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Phe Met His Leu Leu Gln Arg His Leu Asp Asp Leu Leu Asp Ser Asn
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Asp Leu Glu Phe Ile Arg Ser Phe Phe Ala Asn Ile Glu Gln Gly Leu
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Tyr Lys Asp Leu Trp Glu Arg Val Leu Asp Val Ala Tyr Glu Ala Lys
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Ile Phe Ser Leu Pro Ile Thr Arg Lys Lys Met Met Leu Ile Lys Glu
485 490 495

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Val Val Asn Ser Pro Lys Lys Pro Val Glu Ser Lys Ser Leu Thr Thr
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Asp Lys Ile Ile Val Gly Phe Gly Glu Glu Thr Asn Leu Ile Leu Arg
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Lys Leu Thr Ser Gly Pro Ala Asp Leu Asp Val Ile Ser Ile Ile Gly
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Met Pro Gly Leu Gly Lys Thr Thr Leu Ala Tyr Lys Val Tyr Asn Asp
565 570 575

Lys Ser Val Ser Ser His Phe Asp Leu Arg Ala Trp Cys Thr Val Asp
580 585 590

Gln Val Tyr Asp Glu Lys Lys Leu Leu Asp Lys Ile Phe Asn Gln Val
595 600 605

Ser Asp Ser Asn Ser Lys Leu Ser Glu Asn Ile Asp Val Ala Asp Lys
610 615 620

Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr Leu Ile Val Leu Asp Asp
625 630 635 640

Val Trp Asp Thr Asn Thr Trp Asp Glu Leu Thr Arg Pro Phe Pro Asp
645 650 655

Gly Met Lys Gly Ser Arg Ile Ile Leu Thr Thr Arg Glu Lys Lys Val
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Ala Leu His Gly Lys Leu Tyr Thr Asp Pro Leu Asn Leu Arg Leu Leu
675 680 685

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690 695 700

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705 710 715 720

Asn Cys Lys Gly Leu Pro Leu Val Val Asp Leu Ile Ala Gly Ile Ile
725 730 735

Ala Gly Arg Glu Lys Lys Lys Ser Val Trp Leu Glu Val Val Asn Asn
740 745 750

Leu His Ser Phe Ile Leu Lys Asn Glu Val Glu Val Met Lys Val Ile
755 760 765

Glu Ile Ser Tyr Asp His Leu Pro Asp His Leu Lys Pro Cys Leu Leu
770 775 780

Tyr Phe Ala Ser Ala Pro Lys Asp Trp Val Thr Thr Ile His Glu Leu
785 790 795 800

Lys Leu Ile Trp Gly Phe Glu Gly Phe Val Glu Lys Thr Asp Met Lys
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Ser Leu Glu Glu Val Val Lys Ile Tyr Leu Asp Asp Leu Ile Ser Ser
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Ser Leu Val Ile Cys Phe Asn Glu Ile Gly Asp Tyr Pro Thr Cys Gln
835 840 845

Leu His Asp Leu Val His Asp Phe Cys Leu Ile Lys Ala Arg Lys Glu
850 855 860

Lys Leu Cys Asp Arg Ile Ser Ser Ser Ala Pro Ser Asp Leu Leu Pro
865 870 875 880

Arg Gln Ile Ser Ile Asp Tyr Asp Asp Asp Glu Glu His Phe Gly Leu
885 890 895

Asn Phe Val Leu Phe Gly Ser Asn Lys Lys Arg His Ser Gly Lys His
900 905 910

Leu Tyr Ser Leu Thr Ile Asn Gly Asp Glu Leu Asp Asp His Leu Ser
915 920 925

Asp Thr Phe His Leu Arg His Leu Arg Leu Leu Arg Thr Leu His Leu
930 935 940

Glu Ser Ser Phe Ile Met Val Lys Asp Ser Leu Leu Asn Glu Ile Cys
945 950 955 960

Met Leu Asn His Leu Arg Tyr Leu Ser Ile Gly Thr Glu Val Lys Ser
965 970 975

Leu Pro Leu Ser Phe Ser Asn Leu Trp Asn Leu Glu Ile Leu Phe Val
980 985 990

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995 1000 1005

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Trp Phe Pro Lys Leu Asp Phe Leu Thr Glu Leu Glu Lys Leu Thr
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Val Asp Phe Glu Arg Ser Asn Thr Asn Asp Ser Gly Ser Ser Ala
1100 1105 1110

Ala Ile Asn Arg Pro Trp Asp Phe His Phe Pro Ser Ser Leu Lys
1115 1120 1125

Arg Leu Gln Leu His Glu Phe Pro Leu Thr Ser Asp Ser Leu Ser
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<222> (1)..(7967)

<223> Sequence of the 7967 bp Sau3AI genomic DNA fragment of ARD 1197-1.6 BAC 211 present in p211F-C12, Rpi-blb2 gene including natural regulatory elements necessary for correct expression of the gene. The initiation codon (ATG position 1546-1548) and the termination codon (TAG position 5433-5435)

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<222> (5433)..(5435)

<223> Sequence of the 7967 bp Sau3AI genomic DNA fragment of ARD 1197-1 6 BAC 211 present in p211F-C12, Rpi-blb2 gene including natural r egulatory elements necessary for correct expression of the gene. The initiation codon (ATG position 1546-1548) and the termination codon (TAG position 5433-5435)

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<223> Sequence of the 7967 bp Sau3AI genomic DNA fragment of ARD 1197-1 6 BAC 211 present in p211F-C12, Rpi-blb2 gene including natural r egulatory elements necessary for correct expression of the gene. The initiation codon (ATG position 1546-1548) and the termination codon (TAG position 5433-5435)

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<223> Sequence of 9949 bp Sau3AI genomic DNA fragment of S. bulbocastanum 2002 BAC BlbSP39 present in pSP39-20. The genomic fragment harbours the Rpi-blb2 gene including natural elements necessary for expression. Iinitiation codon (ATG position 1413-1415), the termination codon (TAG position 5300-5303)

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<223> Sequence of 9949 bp Sau3AI genomic DNA fragment of S. bulbocastanum 2002 BAC BlbSP39 present in pSP39-20. The genomic fragment harbours the Rpi-blb2 gene including natural elements necessary for expression. Iinitiation codon (ATG position 1413-1415), the termination codon (TAG position 5300-5303)

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Asp Phe Glu Gln Phe Glu Asp Ile Met Thr Arg Lys Arg Gln Glu Val
65                  70                  75                  80

gag aat ctg ctt caa cca ctt ttg gat gat gat gtc ttt act agc ctc     288
Glu Asn Leu Leu Gln Pro Leu Leu Asp Asp Asp Val Phe Thr Ser Leu
                85                  90                  95

acc agt aat atg gat gac tgt atc agc ttg tat cat cgt tct tat aaa     336
Thr Ser Asn Met Asp Asp Cys Ile Ser Leu Tyr His Arg Ser Tyr Lys
            100                 105                 110
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tca gat gcc atc atg atg gat gag caa ttg gac ttc ctc ctc ttg aat      384
Ser Asp Ala Ile Met Met Asp Glu Gln Leu Asp Phe Leu Leu Leu Asn
        115                 120                 125

ctc tat cat cta tcc aag cat cac gct gaa aag ata ttt cct gga gtg      432
Leu Tyr His Leu Ser Lys His His Ala Glu Lys Ile Phe Pro Gly Val
    130                 135                 140

act caa tat gaa gtt ctt cag aat ata tgt ggc aac ata aga gat ttc      480
Thr Gln Tyr Glu Val Leu Gln Asn Ile Cys Gly Asn Ile Arg Asp Phe
145                 150                 155                 160

cat ggg ttg ata gtg aat ggt tgc att aag cat gag atg gtt gag aat      528
His Gly Leu Ile Val Asn Gly Cys Ile Lys His Glu Met Val Glu Asn
                165                 170                 175

gtc ttr cct ctg ttt caa ctc atg gct gac aga gta gga cac ttc ctt      576
Val Xaa Pro Leu Phe Gln Leu Met Ala Asp Arg Val Gly His Phe Leu
            180                 185                 190

tgg gat gat cag act gat gaa gac tct cga ctc tcc gag cta gat gag      624
Trp Asp Asp Gln Thr Asp Glu Asp Ser Arg Leu Ser Glu Leu Asp Glu
        195                 200                 205

gat gaa caa aat gat aga gac tct cga ctt ttc aag cta gca cat cta      672
Asp Glu Gln Asn Asp Arg Asp Ser Arg Leu Phe Lys Leu Ala His Leu
    210                 215                 220

ctc ttg aag atc gtt ccg gtt gaa ctg gag gtt ata cac ata tgt tat      720
Leu Leu Lys Ile Val Pro Val Glu Leu Glu Val Ile His Ile Cys Tyr
225                 230                 235                 240

aca aac ttg aaa gct tca act tca gct gaa gtt gga ctc ttc att aag      768
Thr Asn Leu Lys Ala Ser Thr Ser Ala Glu Val Gly Leu Phe Ile Lys
                245                 250                 255

cag ctt cta gaa acc tct cca gat att ctg agg gaa tat cta att cct      816
Gln Leu Leu Glu Thr Ser Pro Asp Ile Leu Arg Glu Tyr Leu Ile Pro
            260                 265                 270

ctg caa gag cac atg gta act gtt att acc cct agc act tca ggg gct      864
Leu Gln Glu His Met Val Thr Val Ile Thr Pro Ser Thr Ser Gly Ala
        275                 280                 285

cga aac att cat gtc atg atg gaa ttc cta tta ctt att ctt tct gat      912
Arg Asn Ile His Val Met Met Glu Phe Leu Leu Leu Ile Leu Ser Asp
    290                 295                 300

atg ccc aag gac ttt att cat cat gac aaa ctt ttt gat ctc ttg gat      960
Met Pro Lys Asp Phe Ile His His Asp Lys Leu Phe Asp Leu Leu Asp
305                 310                 315                 320

cgt gtc gga gta ctt acc agg gag gta tca act ctt gta cgt gac ttg     1008
Arg Val Gly Val Leu Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu
                325                 330                 335

gaa gag gaa cca agg aat aaa gag ggt aat aac caa aca aat tgt gca     1056
Glu Glu Glu Pro Arg Asn Lys Glu Gly Asn Asn Gln Thr Asn Cys Ala
            340                 345                 350

acc cta gac ttg ctg gaa aat att gaa ctc ctc aag aaa gat ctc aaa     1104
Thr Leu Asp Leu Leu Glu Asn Ile Glu Leu Leu Lys Lys Asp Leu Lys
        355                 360                 365

cat gtt tat ctg aaa gcc ctg gat tca tct caa tgt tgc ttc ccc atg     1152
His Val Tyr Leu Lys Ala Leu Asp Ser Ser Gln Cys Cys Phe Pro Met
    370                 375                 380

agt gat gga cca ctc ttc atg cat ctt cta cac ata cac tta aat gat     1200
Ser Asp Gly Pro Leu Phe Met His Leu Leu His Ile His Leu Asn Asp
385                 390                 395                 400

ttg tta gat tct aat gct tat tca att gct ttg ata aag gaa gaa atc     1248
```

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Leu Leu Asp Ser Asn Ala Tyr Ser Ile Ala Leu Ile Lys Glu Glu Ile
                405                 410                 415

gag ctg gtg aag caa gac ctg aaa ttc ata aga tca ttc ttt gtg gat  1296
Glu Leu Val Lys Gln Asp Leu Lys Phe Ile Arg Ser Phe Phe Val Asp
            420                 425                 430

gct gag caa gga ttg tat aaa gat ctc tgg gca cgt gtt cta gat gtg  1344
Ala Glu Gln Gly Leu Tyr Lys Asp Leu Trp Ala Arg Val Leu Asp Val
        435                 440                 445

gct tat gag gca aaa gat gtc ata gat tca att att gtt cga gat aat  1392
Ala Tyr Glu Ala Lys Asp Val Ile Asp Ser Ile Ile Val Arg Asp Asn
    450                 455                 460

ggt ctc tta cat ctt att ttc tca ctt ccc att acc ata aag aag atc  1440
Gly Leu Leu His Leu Ile Phe Ser Leu Pro Ile Thr Ile Lys Lys Ile
465                 470                 475                 480

aaa ctt atc aaa gaa gag atc tct gct tta gat gag aac att ccc aag  1488
Lys Leu Ile Lys Glu Glu Ile Ser Ala Leu Asp Glu Asn Ile Pro Lys
                485                 490                 495

gac aga ggt cta atc gtt gtg aac tct ccc aag aaa cca gtt gag aga  1536
Asp Arg Gly Leu Ile Val Val Asn Ser Pro Lys Lys Pro Val Glu Arg
            500                 505                 510

aag tca ttg aca act gat aaa ata act gta ggt ttt gag gag gaa aca  1584
Lys Ser Leu Thr Thr Asp Lys Ile Thr Val Gly Phe Glu Glu Glu Thr
        515                 520                 525

aac ttg ata ctt aga aag ctc acc agt gga tcg gca gat cta gat gtc  1632
Asn Leu Ile Leu Arg Lys Leu Thr Ser Gly Ser Ala Asp Leu Asp Val
    530                 535                 540

att tcg atc act ggt atg ccg ggt tca ggt aaa act act ttg gca tac  1680
Ile Ser Ile Thr Gly Met Pro Gly Ser Gly Lys Thr Thr Leu Ala Tyr
545                 550                 555                 560

aaa gta tac aat gat aag tca gtt tct agc cgt ttc gac ctt cgt gca  1728
Lys Val Tyr Asn Asp Lys Ser Val Ser Ser Arg Phe Asp Leu Arg Ala
                565                 570                 575

tgg tgc acg gtc gac caa gga tgt gat gag aag aag ttg ttg aat aca  1776
Trp Cys Thr Val Asp Gln Gly Cys Asp Glu Lys Lys Leu Leu Asn Thr
            580                 585                 590

att ttc agt caa gtt agt gac tca gat tca aaa ttg agt gag aat att  1824
Ile Phe Ser Gln Val Ser Asp Ser Asp Ser Lys Leu Ser Glu Asn Ile
        595                 600                 605

gat gtt gct gat aaa tta cgg aaa caa ctg ttt gga aag agg tat ctt  1872
Asp Val Ala Asp Lys Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr Leu
    610                 615                 620

att gtc tta gat gac gtg tgg gat act act aca tgg gat gag tta aca  1920
Ile Val Leu Asp Asp Val Trp Asp Thr Thr Thr Trp Asp Glu Leu Thr
625                 630                 635                 640

aga cct ttt cct gaa tct aag aaa gga agt agg att att ttg aca act  1968
Arg Pro Phe Pro Glu Ser Lys Lys Gly Ser Arg Ile Ile Leu Thr Thr
                645                 650                 655

cgg gaa aag gaa gtg gct ttg cat gga aag ctg aac act gat cct ctt  2016
Arg Glu Lys Glu Val Ala Leu His Gly Lys Leu Asn Thr Asp Pro Leu
            660                 665                 670

gac ctt cga ttg cta aga cca gat gaa agt tgg gaa cta tta gag aaa  2064
Asp Leu Arg Leu Leu Arg Pro Asp Glu Ser Trp Glu Leu Leu Glu Lys
        675                 680                 685

agg gca ttt ggg aat gag agt tgc cct gat gaa cta tta gat gtc ggt  2112
Arg Ala Phe Gly Asn Glu Ser Cys Pro Asp Glu Leu Leu Asp Val Gly
```

690 695 700

aaa gaa ata gcc gaa aat tgt aaa ggg ctt cct ttg gtg gct gat ctg 2160
Lys Glu Ile Ala Glu Asn Cys Lys Gly Leu Pro Leu Val Ala Asp Leu
705 710 715 720

att gct gga gtc att gct ggg agg gaa aag aaa agg agt gtg tgg ctt 2208
Ile Ala Gly Val Ile Ala Gly Arg Glu Lys Lys Arg Ser Val Trp Leu
725 730 735

gaa gtt caa agt agt ttg agt tct ttt att ttg aac agt gaa gtg gaa 2256
Glu Val Gln Ser Ser Leu Ser Ser Phe Ile Leu Asn Ser Glu Val Glu
740 745 750

gtg atg aaa gtt ata gaa tta agt tat gac cat tta cca cat cac ctc 2304
Val Met Lys Val Ile Glu Leu Ser Tyr Asp His Leu Pro His His Leu
755 760 765

aag cca tgc ttg ctg tat ttt gca agt ttt ccg aag gac act tca ttg 2352
Lys Pro Cys Leu Leu Tyr Phe Ala Ser Phe Pro Lys Asp Thr Ser Leu
770 775 780

aca atc tat gag ttg aat gtt tat ttc ggt gct gaa gga ttt gtg gga 2400
Thr Ile Tyr Glu Leu Asn Val Tyr Phe Gly Ala Glu Gly Phe Val Gly
785 790 795 800

aag acg gag atg aac agt atg gaa gaa gtg gtg aag att tat atg gat 2448
Lys Thr Glu Met Asn Ser Met Glu Glu Val Val Lys Ile Tyr Met Asp
805 810 815

gat tta att tac agt agc ttg gta att tgt ttc aat gag ata ggt tat 2496
Asp Leu Ile Tyr Ser Ser Leu Val Ile Cys Phe Asn Glu Ile Gly Tyr
820 825 830

gca ctg aat ttc caa att cat gat ctt gtg cat gac ttt tgt ttg ata 2544
Ala Leu Asn Phe Gln Ile His Asp Leu Val His Asp Phe Cys Leu Ile
835 840 845

aaa gca aga aag gaa aat ttg ttt gat cag ata aga tca agt gct cca 2592
Lys Ala Arg Lys Glu Asn Leu Phe Asp Gln Ile Arg Ser Ser Ala Pro
850 855 860

tca gat ttg ttg cct cgt caa att acc att gat tgt gat gag gag gag 2640
Ser Asp Leu Leu Pro Arg Gln Ile Thr Ile Asp Cys Asp Glu Glu Glu
865 870 875 880

cac ttt ggg ctt aat ttt gtc atg ttc gat tca aat aag aaa agg cat 2688
His Phe Gly Leu Asn Phe Val Met Phe Asp Ser Asn Lys Lys Arg His
885 890 895

tct ggt aaa cac ctc tat tct ttg agg ata att gga gac cag ctg gat 2736
Ser Gly Lys His Leu Tyr Ser Leu Arg Ile Ile Gly Asp Gln Leu Asp
900 905 910

gac agt gtt tct gat gca ttt cac cta aga cac ttg agg ctt ctt aga 2784
Asp Ser Val Ser Asp Ala Phe His Leu Arg His Leu Arg Leu Leu Arg
915 920 925

gtg ttg gac ctg cat acg tct ttt atc atg gtg aaa gat tct ttg ctg 2832
Val Leu Asp Leu His Thr Ser Phe Ile Met Val Lys Asp Ser Leu Leu
930 935 940

aat gaa ata tgc atg ttg aat cat ttg agg tac tta tcc att gac aca 2880
Asn Glu Ile Cys Met Leu Asn His Leu Arg Tyr Leu Ser Ile Asp Thr
945 950 955 960

caa gtt aaa tat ctg cct ttg tct ttc tca aac ctc tgg aat cta gaa 2928
Gln Val Lys Tyr Leu Pro Leu Ser Phe Ser Asn Leu Trp Asn Leu Glu
965 970 975

agc ctg ttt gtg tct acc aac aga tca atc ttg gta cta tta ccg aga 2976
Ser Leu Phe Val Ser Thr Asn Arg Ser Ile Leu Val Leu Leu Pro Arg
980 985 990

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att ttg gat ctt gta aag ttg cga  gtg ctg tcc gtg gat  gct tgt tct   3024
Ile Leu Asp Leu Val Lys Leu Arg  Val Leu Ser Val Asp  Ala Cys Ser
       995                 1000                1005

ttc ttt  gat atg gat gca gat  gaa tca ata ttg ata  gca gag gac      3069
Phe Phe  Asp Met Asp Ala Asp  Glu Ser Ile Leu Ile  Ala Glu Asp
    1010                1015                1020

aca aag  tta gag aac ttg aga  ata tta acg gaa ctg  ttg att tcc      3114
Thr Lys  Leu Glu Asn Leu Arg  Ile Leu Thr Glu Leu  Leu Ile Ser
    1025                1030                1035

tat tcg  aaa gat aca aag aat  att ttc aaa agg ttt  ccc aat ctt      3159
Tyr Ser  Lys Asp Thr Lys Asn  Ile Phe Lys Arg Phe  Pro Asn Leu
    1040                1045                1050

cag ttg  ctt tca ttt gaa ctc  aag gag tca tgg gat  tat tca aca      3204
Gln Leu  Leu Ser Phe Glu Leu  Lys Glu Ser Trp Asp  Tyr Ser Thr
    1055                1060                1065

gag caa  cat tgg ttc tcg gaa  ttg gat ttc cta act  gaa cta gaa      3249
Glu Gln  His Trp Phe Ser Glu  Leu Asp Phe Leu Thr  Glu Leu Glu
    1070                1075                1080

aca ctc  tct gta ggt ttt aaa  agt tca aac aca aac  gat agt ggg      3294
Thr Leu  Ser Val Gly Phe Lys  Ser Ser Asn Thr Asn  Asp Ser Gly
    1085                1090                1095

tcc tct  gta gcg aca aat cgg  ccg tgg gat ttt cac  ttc cct tca      3339
Ser Ser  Val Ala Thr Asn Arg  Pro Trp Asp Phe His  Phe Pro Ser
    1100                1105                1110

aat ttg  aaa ata ctg tgg ttg  cgt gaa ttt ccg ctg  aca tcc gat      3384
Asn Leu  Lys Ile Leu Trp Leu  Arg Glu Phe Pro Leu  Thr Ser Asp
    1115                1120                1125

tca cta  tca aca ata gcg aga  ctg ccc aac ctt gaa  gag ttg tcc      3429
Ser Leu  Ser Thr Ile Ala Arg  Leu Pro Asn Leu Glu  Glu Leu Ser
    1130                1135                1140

ctt tat  cat aca atc atc cat  gga gaa gaa tgg aac  atg ggg gag      3474
Leu Tyr  His Thr Ile Ile His  Gly Glu Glu Trp Asn  Met Gly Glu
    1145                1150                1155

gaa gac  acc ttt gag aat ctc  aaa ttt ttg aac ttc  aat caa gtt      3519
Glu Asp  Thr Phe Glu Asn Leu  Lys Phe Leu Asn Phe  Asn Gln Val
    1160                1165                1170

agt att  tcc aag tgg gag gtt  gga gag gaa tcc ttc  ccc aat ctt      3564
Ser Ile  Ser Lys Trp Glu Val  Gly Glu Glu Ser Phe  Pro Asn Leu
    1175                1180                1185

gag aaa  tta aaa ctg cgg gga  tgt cat aag cta gag  gag att cca      3609
Glu Lys  Leu Lys Leu Arg Gly  Cys His Lys Leu Glu  Glu Ile Pro
    1190                1195                1200

cct agt  ttt gga gat att tat  tca ttg aaa tct atc  aaa att gta      3654
Pro Ser  Phe Gly Asp Ile Tyr  Ser Leu Lys Ser Ile  Lys Ile Val
    1205                1210                1215

aag agt  cct caa ctt gaa gat  tct gct ctc aaa att  aag gaa tac      3699
Lys Ser  Pro Gln Leu Glu Asp  Ser Ala Leu Lys Ile  Lys Glu Tyr
    1220                1225                1230

gct gaa  gat atg agg gga ggg  gac gag ctt cag atc  ctt ggc caa      3744
Ala Glu  Asp Met Arg Gly Gly  Asp Glu Leu Gln Ile  Leu Gly Gln
    1235                1240                1245

aag aat  atc ccc tta ttt aag  tag                                  3768
Lys Asn  Ile Pro Leu Phe Lys
    1250                1255
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<210> 8

<211> 1255

<212> PRT

<213> Lycopersicon lycopersicum

<220>

<221> misc_feature

<222> (178)..(178)

<223> The 'Xaa' at location 178 stands for Leu.

<400> 8

Met Glu Lys Arg Lys Asp Asn Glu Glu Ala Asn Asn Ser Leu Val Leu
1 5 10 15

Phe Ser Ala Leu Ser Lys Asp Ile Ala Asp Val Leu Val Phe Leu Glu
20 25 30

Asn Glu Glu Asn Gln Lys Ala Leu Asp Lys Asp Gln Val Glu Lys Ile
35 40 45

Lys Leu Lys Met Ala Phe Ile Cys Thr Tyr Val Gln Leu Ser Cys Ser
50 55 60

Asp Phe Glu Gln Phe Glu Asp Ile Met Thr Arg Lys Arg Gln Glu Val
65 70 75 80

Glu Asn Leu Leu Gln Pro Leu Leu Asp Asp Asp Val Phe Thr Ser Leu
85 90 95

Thr Ser Asn Met Asp Asp Cys Ile Ser Leu Tyr His Arg Ser Tyr Lys
100 105 110

Ser Asp Ala Ile Met Met Asp Glu Gln Leu Asp Phe Leu Leu Leu Asn
115 120 125

Leu Tyr His Leu Ser Lys His His Ala Glu Lys Ile Phe Pro Gly Val
130 135 140

Thr Gln Tyr Glu Val Leu Gln Asn Ile Cys Gly Asn Ile Arg Asp Phe
145 150 155 160

His Gly Leu Ile Val Asn Gly Cys Ile Lys His Glu Met Val Glu Asn
165 170 175

Val Xaa Pro Leu Phe Gln Leu Met Ala Asp Arg Val Gly His Phe Leu
180 185 190

Trp Asp Asp Gln Thr Asp Glu Asp Ser Arg Leu Ser Glu Leu Asp Glu
195 200 205

Asp Glu Gln Asn Asp Arg Asp Ser Arg Leu Phe Lys Leu Ala His Leu
210 215 220

Leu Leu Lys Ile Val Pro Val Glu Leu Glu Val Ile His Ile Cys Tyr
225 230 235 240

Thr Asn Leu Lys Ala Ser Thr Ser Ala Glu Val Gly Leu Phe Ile Lys
245 250 255

Gln Leu Leu Glu Thr Ser Pro Asp Ile Leu Arg Glu Tyr Leu Ile Pro
260 265 270

Leu Gln Glu His Met Val Thr Val Ile Thr Pro Ser Thr Ser Gly Ala
275 280 285

Arg Asn Ile His Val Met Met Glu Phe Leu Leu Leu Ile Leu Ser Asp
290 295 300

Met Pro Lys Asp Phe Ile His His Asp Lys Leu Phe Asp Leu Leu Asp
305 310 315 320

Arg Val Gly Val Leu Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu
325 330 335

Glu Glu Glu Pro Arg Asn Lys Glu Gly Asn Asn Gln Thr Asn Cys Ala
340 345 350

Thr Leu Asp Leu Leu Glu Asn Ile Glu Leu Leu Lys Lys Asp Leu Lys
355 360 365

His Val Tyr Leu Lys Ala Leu Asp Ser Ser Gln Cys Cys Phe Pro Met
370 375 380

Ser Asp Gly Pro Leu Phe Met His Leu Leu His Ile His Leu Asn Asp
385 390 395 400

Leu Leu Asp Ser Asn Ala Tyr Ser Ile Ala Leu Ile Lys Glu Glu Ile
405 410 415

Glu Leu Val Lys Gln Asp Leu Lys Phe Ile Arg Ser Phe Phe Val Asp
420 425 430

Ala Glu Gln Gly Leu Tyr Lys Asp Leu Trp Ala Arg Val Leu Asp Val
435 440 445

Ala Tyr Glu Ala Lys Asp Val Ile Asp Ser Ile Ile Val Arg Asp Asn
450 455 460

Gly Leu Leu His Leu Ile Phe Ser Leu Pro Ile Thr Ile Lys Lys Ile
465 470 475 480

Lys Leu Ile Lys Glu Glu Ile Ser Ala Leu Asp Glu Asn Ile Pro Lys
485 490 495

Asp Arg Gly Leu Ile Val Val Asn Ser Pro Lys Lys Pro Val Glu Arg

500 505 510

Lys Ser Leu Thr Thr Asp Lys Ile Thr Val Gly Phe Glu Glu Glu Thr
515 520 525

Asn Leu Ile Leu Arg Lys Leu Thr Ser Gly Ser Ala Asp Leu Asp Val
530 535 540

Ile Ser Ile Thr Gly Met Pro Gly Ser Gly Lys Thr Thr Leu Ala Tyr
545 550 555 560

Lys Val Tyr Asn Asp Lys Ser Val Ser Ser Arg Phe Asp Leu Arg Ala
565 570 575

Trp Cys Thr Val Asp Gln Gly Cys Asp Glu Lys Lys Leu Leu Asn Thr
580 585 590

Ile Phe Ser Gln Val Ser Asp Ser Asp Ser Lys Leu Ser Glu Asn Ile
595 600 605

Asp Val Ala Asp Lys Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr Leu
610 615 620

Ile Val Leu Asp Asp Val Trp Asp Thr Thr Thr Trp Asp Glu Leu Thr
625 630 635 640

Arg Pro Phe Pro Glu Ser Lys Lys Gly Ser Arg Ile Ile Leu Thr Thr
645 650 655

Arg Glu Lys Glu Val Ala Leu His Gly Lys Leu Asn Thr Asp Pro Leu
660 665 670

Asp Leu Arg Leu Leu Arg Pro Asp Glu Ser Trp Glu Leu Leu Glu Lys
675 680 685

Arg Ala Phe Gly Asn Glu Ser Cys Pro Asp Glu Leu Leu Asp Val Gly
690 695 700

Lys Glu Ile Ala Glu Asn Cys Lys Gly Leu Pro Leu Val Ala Asp Leu
705 710 715 720

Ile Ala Gly Val Ile Ala Gly Arg Glu Lys Lys Arg Ser Val Trp Leu
725 730 735

Glu Val Gln Ser Ser Leu Ser Ser Phe Ile Leu Asn Ser Glu Val Glu
740 745 750

Val Met Lys Val Ile Glu Leu Ser Tyr Asp His Leu Pro His His Leu
755 760 765

Lys Pro Cys Leu Leu Tyr Phe Ala Ser Phe Pro Lys Asp Thr Ser Leu
770 775 780

Thr Ile Tyr Glu Leu Asn Val Tyr Phe Gly Ala Glu Gly Phe Val Gly
785 790 795 800

Lys Thr Glu Met Asn Ser Met Glu Glu Val Val Lys Ile Tyr Met Asp
805 810 815

Asp Leu Ile Tyr Ser Ser Leu Val Ile Cys Phe Asn Glu Ile Gly Tyr
820 825 830

Ala Leu Asn Phe Gln Ile His Asp Leu Val His Asp Phe Cys Leu Ile
835 840 845

Lys Ala Arg Lys Glu Asn Leu Phe Asp Gln Ile Arg Ser Ser Ala Pro
850 855 860

Ser Asp Leu Leu Pro Arg Gln Ile Thr Ile Asp Cys Asp Glu Glu Glu
865 870 875 880

His Phe Gly Leu Asn Phe Val Met Phe Asp Ser Asn Lys Lys Arg His
885 890 895

Ser Gly Lys His Leu Tyr Ser Leu Arg Ile Ile Gly Asp Gln Leu Asp
900 905 910

Asp Ser Val Ser Asp Ala Phe His Leu Arg His Leu Arg Leu Leu Arg
915 920 925

Val Leu Asp Leu His Thr Ser Phe Ile Met Val Lys Asp Ser Leu Leu
930 935 940

Asn Glu Ile Cys Met Leu Asn His Leu Arg Tyr Leu Ser Ile Asp Thr
945 950 955 960

Gln Val Lys Tyr Leu Pro Leu Ser Phe Ser Asn Leu Trp Asn Leu Glu
965 970 975

Ser Leu Phe Val Ser Thr Asn Arg Ser Ile Leu Val Leu Leu Pro Arg
980 985 990

Ile Leu Asp Leu Val Lys Leu Arg Val Leu Ser Val Asp Ala Cys Ser
995 1000 1005

Phe Phe Asp Met Asp Ala Asp Glu Ser Ile Leu Ile Ala Glu Asp
1010 1015 1020

Thr Lys Leu Glu Asn Leu Arg Ile Leu Thr Glu Leu Leu Ile Ser
1025 1030 1035

Tyr Ser Lys Asp Thr Lys Asn Ile Phe Lys Arg Phe Pro Asn Leu
1040 1045 1050

Gln Leu Leu Ser Phe Glu Leu Lys Glu Ser Trp Asp Tyr Ser Thr
1055 1060 1065

Glu Gln His Trp Phe Ser Glu Leu Asp Phe Leu Thr Glu Leu Glu
1070 1075 1080

Thr Leu Ser Val Gly Phe Lys Ser Ser Asn Thr Asn Asp Ser Gly
1085 1090 1095

Ser Ser Val Ala Thr Asn Arg Pro Trp Asp Phe His Phe Pro Ser
1100 1105 1110

Asn Leu Lys Ile Leu Trp Leu Arg Glu Phe Pro Leu Thr Ser Asp
1115 1120 1125

Ser Leu Ser Thr Ile Ala Arg Leu Pro Asn Leu Glu Glu Leu Ser
1130 1135 1140

Leu Tyr His Thr Ile Ile His Gly Glu Glu Trp Asn Met Gly Glu
1145 1150 1155

Glu Asp Thr Phe Glu Asn Leu Lys Phe Leu Asn Phe Asn Gln Val
1160 1165 1170

Ser Ile Ser Lys Trp Glu Val Gly Glu Glu Ser Phe Pro Asn Leu
1175 1180 1185

Glu Lys Leu Lys Leu Arg Gly Cys His Lys Leu Glu Glu Ile Pro
1190 1195 1200

Pro Ser Phe Gly Asp Ile Tyr Ser Leu Lys Ser Ile Lys Ile Val
1205 1210 1215

Lys Ser Pro Gln Leu Glu Asp Ser Ala Leu Lys Ile Lys Glu Tyr
1220 1225 1230

Ala Glu Asp Met Arg Gly Gly Asp Glu Leu Gln Ile Leu Gly Gln
1235 1240 1245

Lys Asn Ile Pro Leu Phe Lys
1250 1255

<210> 9

<211> 3774

<212> DNA

<213> Lycopersicon lycopersicum

<220>

<221> CDS

<222> (1)..(3774)

<223> Mi1.2 from tomato

<400> 9

atg gaa aaa cga aaa gat att gaa gaa gca aac aac tca ttg gtg tta 48
Met Glu Lys Arg Lys Asp Ile Glu Glu Ala Asn Asn Ser Leu Val Leu
1 5 10 15

```
ttt tct gct ctt agc aag gac att gcc aat gtt cta att ttc cta gag      96
Phe Ser Ala Leu Ser Lys Asp Ile Ala Asn Val Leu Ile Phe Leu Glu
            20                  25                  30

aat gag gaa aat caa aaa gct ctt gac aaa gat caa gtt gaa aag cta     144
Asn Glu Glu Asn Gln Lys Ala Leu Asp Lys Asp Gln Val Glu Lys Leu
        35                  40                  45

aaa ttg aaa atg gca ttt att tgt aca tat gtt cag ctt tct tat tcc     192
Lys Leu Lys Met Ala Phe Ile Cys Thr Tyr Val Gln Leu Ser Tyr Ser
    50                  55                  60

gat ttt gag cag ttt gaa gat ata atg act aga aat aga caa gag gtt     240
Asp Phe Glu Gln Phe Glu Asp Ile Met Thr Arg Asn Arg Gln Glu Val
65                  70                  75                  80

gag aat ctg ctt caa tca ctt ttg gat gat gat gtc ctt act agc ctc     288
Glu Asn Leu Leu Gln Ser Leu Leu Asp Asp Asp Val Leu Thr Ser Leu
                85                  90                  95

acc agt aat atg gat gac tgt atc agc ttg tat cat cgt tct tat aaa     336
Thr Ser Asn Met Asp Asp Cys Ile Ser Leu Tyr His Arg Ser Tyr Lys
            100                 105                 110

tca gat gcc atc atg atg gat gag caa ttg gac ttc ctc ctc ttg aat     384
Ser Asp Ala Ile Met Met Asp Glu Gln Leu Asp Phe Leu Leu Leu Asn
        115                 120                 125

ctg tat cat cta tcc aag cat cac gct gaa aag ata ttt cct gga gtg     432
Leu Tyr His Leu Ser Lys His His Ala Glu Lys Ile Phe Pro Gly Val
    130                 135                 140

act caa tat gaa gtt ctt cag aat gta tgt ggc aac ata aga gat ttc     480
Thr Gln Tyr Glu Val Leu Gln Asn Val Cys Gly Asn Ile Arg Asp Phe
145                 150                 155                 160

cat ggg ttg ata ctg aat ggt tgc att aag cat gag atg gtt gag aat     528
His Gly Leu Ile Leu Asn Gly Cys Ile Lys His Glu Met Val Glu Asn
                165                 170                 175

gtc tta cct ctg ttt caa ctc atg gct gaa aga gta gga cac ttc ctt     576
Val Leu Pro Leu Phe Gln Leu Met Ala Glu Arg Val Gly His Phe Leu
            180                 185                 190

tgg gag gat cag act gat gaa gac tct cgg ctc tcc gag cta gat gag     624
Trp Glu Asp Gln Thr Asp Glu Asp Ser Arg Leu Ser Glu Leu Asp Glu
        195                 200                 205

gat gaa cac aat gat aga gac tct cga ctc ttc cag cta aca cat cta     672
Asp Glu His Asn Asp Arg Asp Ser Arg Leu Phe Gln Leu Thr His Leu
    210                 215                 220

ctc ttg aag att gtt cca act gaa ctg gag gtt atg cac ata tgt tat     720
Leu Leu Lys Ile Val Pro Thr Glu Leu Glu Val Met His Ile Cys Tyr
225                 230                 235                 240

aca aat ttg aaa gct tca act tca gca gaa gtt gga cgc ttc att aag     768
Thr Asn Leu Lys Ala Ser Thr Ser Ala Glu Val Gly Arg Phe Ile Lys
                245                 250                 255

aag ctc ctg gaa acc tca ccg gat att ctc aga gaa tat atc att caa     816
Lys Leu Leu Glu Thr Ser Pro Asp Ile Leu Arg Glu Tyr Ile Ile Gln
            260                 265                 270

cta caa gag cat atg tta act gtt att ccc cct agc act tta ggg gct     864
Leu Gln Glu His Met Leu Thr Val Ile Pro Pro Ser Thr Leu Gly Ala
        275                 280                 285

cga aac att cat gtc atg atg gaa ttc cta tta ctt att ctt tct gat     912
Arg Asn Ile His Val Met Met Glu Phe Leu Leu Leu Ile Leu Ser Asp
    290                 295                 300
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atg ccc aag gac ttt att cat cat gac aaa ctt ttt gat ctc ttg gct      960
Met Pro Lys Asp Phe Ile His His Asp Lys Leu Phe Asp Leu Leu Ala
305                 310                 315                 320

cat gtt gga aca ctt acc agg gag gta tcg act ctt gta cgt gac ttg     1008
His Val Gly Thr Leu Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu
                325                 330                 335

gaa gag aaa tta agg aat aaa gag ggt aat aac caa aca aat tgt gca     1056
Glu Glu Lys Leu Arg Asn Lys Glu Gly Asn Asn Gln Thr Asn Cys Ala
            340                 345                 350

acc cta gac ttg ctg gaa aat att gaa ctc ctc aag aaa gat ctc aaa     1104
Thr Leu Asp Leu Leu Glu Asn Ile Glu Leu Leu Lys Lys Asp Leu Lys
        355                 360                 365

cat gtt tat ctg aaa gcc cca aat tca tct caa tgt tgc ttc ccc atg     1152
His Val Tyr Leu Lys Ala Pro Asn Ser Ser Gln Cys Cys Phe Pro Met
    370                 375                 380

agt gat gga cca ctc ttc atg cat ctt cta cac atg cac tta aat gat     1200
Ser Asp Gly Pro Leu Phe Met His Leu Leu His Met His Leu Asn Asp
385                 390                 395                 400

ttg cta gat tct aat gct tat tca att tct ttg ata aag gaa gaa atc     1248
Leu Leu Asp Ser Asn Ala Tyr Ser Ile Ser Leu Ile Lys Glu Glu Ile
                405                 410                 415

gag ttg gtg agt caa gaa ctg gaa ttc ata aga tca ttc ttt ggg gat     1296
Glu Leu Val Ser Gln Glu Leu Glu Phe Ile Arg Ser Phe Phe Gly Asp
            420                 425                 430

gct gct gag caa gga ttg tat aaa gat atc tgg gca cgt gtt cta gat     1344
Ala Ala Glu Gln Gly Leu Tyr Lys Asp Ile Trp Ala Arg Val Leu Asp
        435                 440                 445

gtg gct tat gag gca aaa gat gtc ata gat tca att att gtt cga gat     1392
Val Ala Tyr Glu Ala Lys Asp Val Ile Asp Ser Ile Ile Val Arg Asp
    450                 455                 460

aat ggt ctc tta cat ctt att ttc tca ctt ccc att acc ata aag aag     1440
Asn Gly Leu Leu His Leu Ile Phe Ser Leu Pro Ile Thr Ile Lys Lys
465                 470                 475                 480

atc aaa ctt atc aaa gaa gag atc tct gct tta gat gag aac att ccc     1488
Ile Lys Leu Ile Lys Glu Glu Ile Ser Ala Leu Asp Glu Asn Ile Pro
                485                 490                 495

aag gac aga ggt cta atc gtt gtg aac tct ccc aag aaa cca gtt gag     1536
Lys Asp Arg Gly Leu Ile Val Val Asn Ser Pro Lys Lys Pro Val Glu
            500                 505                 510

aga aag tca ttg aca act gat aaa ata att gta ggt ttt gag gag gag     1584
Arg Lys Ser Leu Thr Thr Asp Lys Ile Ile Val Gly Phe Glu Glu Glu
        515                 520                 525

aca aac ttg ata ctt aga aag ctc acc agt gga ccc gca gat tta gat     1632
Thr Asn Leu Ile Leu Arg Lys Leu Thr Ser Gly Pro Ala Asp Leu Asp
    530                 535                 540

gtc att tcg atc acc ggt atg ccg ggt tca ggt aaa act act ttg gca     1680
Val Ile Ser Ile Thr Gly Met Pro Gly Ser Gly Lys Thr Thr Leu Ala
545                 550                 555                 560

tac aaa gta tac aat gat aag tca gtt tct aga cat ttt gac ctt cgt     1728
Tyr Lys Val Tyr Asn Asp Lys Ser Val Ser Arg His Phe Asp Leu Arg
                565                 570                 575

gca tgg tgc acg gtc gat caa gga tat gac gac aag aag ttg ttg gat     1776
Ala Trp Cys Thr Val Asp Gln Gly Tyr Asp Asp Lys Lys Leu Leu Asp
            580                 585                 590

aca att ttc agt caa gtt agt ggc tca gat tca aat ttg agt gag aat     1824
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Thr Ile Phe Ser Gln Val Ser Gly Ser Asp Ser Asn Leu Ser Glu Asn
595                 600                 605

att gat gtt gct gat aaa ttg cgg aaa caa ctg ttt gga aag agg tat  1872
Ile Asp Val Ala Asp Lys Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr
610                 615                 620

ctt att gtc tta gat gat gtg tgg gat act act aca ttg gat gag ttg  1920
Leu Ile Val Leu Asp Asp Val Trp Asp Thr Thr Thr Leu Asp Glu Leu
625                 630                 635                 640

aca aga cct ttt cct gaa gct aag aaa gga agt agg att att ttg aca  1968
Thr Arg Pro Phe Pro Glu Ala Lys Lys Gly Ser Arg Ile Ile Leu Thr
                645                 650                 655

act cga gaa aag gaa gtg gct ttg cat gga aag ctg aac act gat cct  2016
Thr Arg Glu Lys Glu Val Ala Leu His Gly Lys Leu Asn Thr Asp Pro
            660                 665                 670

ctt gac ctt cga ttg cta aga cca gat gaa agt tgg gaa ctt tta gat  2064
Leu Asp Leu Arg Leu Leu Arg Pro Asp Glu Ser Trp Glu Leu Leu Asp
        675                 680                 685

aaa agg aca ttt ggt aat gag agt tgc cct gat gaa cta tta gat gtc  2112
Lys Arg Thr Phe Gly Asn Glu Ser Cys Pro Asp Glu Leu Leu Asp Val
    690                 695                 700

ggt aaa gaa ata gcc gaa aat tgt aaa ggg ctt cct ttg gtg gct gat  2160
Gly Lys Glu Ile Ala Glu Asn Cys Lys Gly Leu Pro Leu Val Ala Asp
705                 710                 715                 720

ctg att gct gga gtc att gct ggg agg gaa aag aaa agg agt gtg tgg  2208
Leu Ile Ala Gly Val Ile Ala Gly Arg Glu Lys Lys Arg Ser Val Trp
                725                 730                 735

ctt gaa gtt caa agt agt ttg agt tct ttt att ttg aac agt gaa gtg  2256
Leu Glu Val Gln Ser Ser Leu Ser Ser Phe Ile Leu Asn Ser Glu Val
            740                 745                 750

gaa gtg atg aaa gtt ata gaa tta agt tat gac cat tta cca cat cac  2304
Glu Val Met Lys Val Ile Glu Leu Ser Tyr Asp His Leu Pro His His
        755                 760                 765

ctc aag cca tgc ttg ctt cac ttt gca agt tgg ccg aag gac act cct  2352
Leu Lys Pro Cys Leu Leu His Phe Ala Ser Trp Pro Lys Asp Thr Pro
    770                 775                 780

ttg aca atc tat ttg ttt act gtt tat ttg ggt gct gaa gga ttt gtg  2400
Leu Thr Ile Tyr Leu Phe Thr Val Tyr Leu Gly Ala Glu Gly Phe Val
785                 790                 795                 800

gaa aag acg gag atg aag ggt ata gaa gaa gtg gtg aag att tat atg  2448
Glu Lys Thr Glu Met Lys Gly Ile Glu Glu Val Val Lys Ile Tyr Met
                805                 810                 815

gat gat tta att tcc agt agc ttg gta att tgt ttc aat gag ata ggt  2496
Asp Asp Leu Ile Ser Ser Ser Leu Val Ile Cys Phe Asn Glu Ile Gly
            820                 825                 830

gat ata ctg aat ttc caa att cat gat ctt gtg cat gac ttt tgt ttg  2544
Asp Ile Leu Asn Phe Gln Ile His Asp Leu Val His Asp Phe Cys Leu
        835                 840                 845

ata aaa gca aga aag gaa aat ttg ttt gat cgg ata aga tca agt gct  2592
Ile Lys Ala Arg Lys Glu Asn Leu Phe Asp Arg Ile Arg Ser Ser Ala
    850                 855                 860

cca tca gat ttg ttg cct cgt caa att acc att gat tat gat gag gag  2640
Pro Ser Asp Leu Leu Pro Arg Gln Ile Thr Ile Asp Tyr Asp Glu Glu
865                 870                 875                 880

gag gag cac ttt ggg ctt aat ttt gtc atg ttc gat tca aat aag aaa  2688
Glu Glu His Phe Gly Leu Asn Phe Val Met Phe Asp Ser Asn Lys Lys
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                    885                 890                 895

agg cat tct ggt aaa cac ctc tat tct ttg agg ata aat gga gac cag   2736
Arg His Ser Gly Lys His Leu Tyr Ser Leu Arg Ile Asn Gly Asp Gln
            900                 905                 910

ctg gat gac agt gtt tct gat gca ttt cac cta aga cac ttg agg ctt   2784
Leu Asp Asp Ser Val Ser Asp Ala Phe His Leu Arg His Leu Arg Leu
        915                 920                 925

att aga gtg ttg gac ctg gaa ccc tct tta atc atg gtg aat gat tct   2832
Ile Arg Val Leu Asp Leu Glu Pro Ser Leu Ile Met Val Asn Asp Ser
    930                 935                 940

ttg ctg aat gaa ata tgc atg ttg aat cat ttg agg tac tta aga att   2880
Leu Leu Asn Glu Ile Cys Met Leu Asn His Leu Arg Tyr Leu Arg Ile
945                 950                 955                 960

cgg aca caa gtt aaa tat ctg cct ttc tct ttc tca aac ctc tgg aat   2928
Arg Thr Gln Val Lys Tyr Leu Pro Phe Ser Phe Ser Asn Leu Trp Asn
                965                 970                 975

cta gaa agt ctg ttt gtg tct aac aaa gga tca atc ttg gta cta tta   2976
Leu Glu Ser Leu Phe Val Ser Asn Lys Gly Ser Ile Leu Val Leu Leu
            980                 985                 990

ccg aga att ttg gat ctt gta aag  ttg cga gtg ctg tcc  gtg ggt gct   3024
Pro Arg Ile Leu Asp Leu Val Lys  Leu Arg Val Leu Ser  Val Gly Ala
        995                1000                1005

tgt tct  ttc ttt gat atg gat  gca gat gaa tca ata  ttg ata gca      3069
Cys Ser  Phe Phe Asp Met Asp  Ala Asp Glu Ser Ile  Leu Ile Ala
    1010                1015                1020

aag gac  aca aag tta gag aac  ttg aga ata tta ggg  gaa ctg ttg      3114
Lys Asp  Thr Lys Leu Glu Asn  Leu Arg Ile Leu Gly  Glu Leu Leu
1025                1030                1035

att tcc  tat tcg aaa gat aca  atg aat att ttc aaa  agg ttt ccc      3159
Ile Ser  Tyr Ser Lys Asp Thr  Met Asn Ile Phe Lys  Arg Phe Pro
1040                1045                1050

aat ctt  cag gtg ctt cag ttt  gaa ctc aag gag tca  tgg gat tat      3204
Asn Leu  Gln Val Leu Gln Phe  Glu Leu Lys Glu Ser  Trp Asp Tyr
1055                1060                1065

tca aca  gag caa cat tgg ttc  ccg aaa ttg gat tgc  cta act gaa      3249
Ser Thr  Glu Gln His Trp Phe  Pro Lys Leu Asp Cys  Leu Thr Glu
1070                1075                1080

cta gaa  aca ctc tgt gta ggt  ttt aaa agt tca aac  aca aac cac      3294
Leu Glu  Thr Leu Cys Val Gly  Phe Lys Ser Ser Asn  Thr Asn His
1085                1090                1095

tgt ggg  tcc tct gtt gtg aca  aat cgg ccg tgg gat  ttt cac ttc      3339
Cys Gly  Ser Ser Val Val Thr  Asn Arg Pro Trp Asp  Phe His Phe
1100                1105                1110

cct tca  aat ttg aaa gaa ctg  ttg ttg tat gac ttt  cct ctg aca      3384
Pro Ser  Asn Leu Lys Glu Leu  Leu Leu Tyr Asp Phe  Pro Leu Thr
1115                1120                1125

tcc gat  tca cta tca aca ata  gcg aga ctg ccc aac  ctt gaa aat      3429
Ser Asp  Ser Leu Ser Thr Ile  Ala Arg Leu Pro Asn  Leu Glu Asn
1130                1135                1140

ttg tcc  ctt tat gat aca atc  atc cag gga gaa gaa  tgg aac atg      3474
Leu Ser  Leu Tyr Asp Thr Ile  Ile Gln Gly Glu Glu  Trp Asn Met
1145                1150                1155

ggg gag  gaa gac act ttt gag  aat ctc aaa ttt ttg  aac ttg cgt      3519
Gly Glu  Glu Asp Thr Phe Glu  Asn Leu Lys Phe Leu  Asn Leu Arg
1160                1165                1170
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cta ctg act ctt tcc aag tgg gag gtt gga gag gaa tcc ttc ccc 3564
Leu Leu Thr Leu Ser Lys Trp Glu Val Gly Glu Glu Ser Phe Pro
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aat ctt gag aaa tta aaa ctg cag gaa tgt ggt aag ctt gag gag 3609
Asn Leu Glu Lys Leu Lys Leu Gln Glu Cys Gly Lys Leu Glu Glu
    1190                1195                1200

att cca cct agt ttt gga gat att tat tca ttg aaa ttt atc aaa 3654
Ile Pro Pro Ser Phe Gly Asp Ile Tyr Ser Leu Lys Phe Ile Lys
    1205                1210                1215

att gta aag agt cct caa ctt gaa gat tct gct ctc aag att aag 3699
Ile Val Lys Ser Pro Gln Leu Glu Asp Ser Ala Leu Lys Ile Lys
    1220                1225                1230

aaa tac gct gaa gat atg aga gga ggg aac gat ctt cag atc ctt 3744
Lys Tyr Ala Glu Asp Met Arg Gly Gly Asn Asp Leu Gln Ile Leu
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            20                  25                  30

Asn Glu Glu Asn Gln Lys Ala Leu Asp Lys Asp Gln Val Glu Lys Leu
        35                  40                  45

Lys Leu Lys Met Ala Phe Ile Cys Thr Tyr Val Gln Leu Ser Tyr Ser
    50                  55                  60

Asp Phe Glu Gln Phe Glu Asp Ile Met Thr Arg Asn Arg Gln Glu Val
65                  70                  75                  80

Glu Asn Leu Leu Gln Ser Leu Leu Asp Asp Asp Val Leu Thr Ser Leu
                85                  90                  95

Thr Ser Asn Met Asp Asp Cys Ile Ser Leu Tyr His Arg Ser Tyr Lys
            100                 105                 110

Ser Asp Ala Ile Met Met Asp Glu Gln Leu Asp Phe Leu Leu Leu Asn
        115                 120                 125

Leu Tyr His Leu Ser Lys His His Ala Glu Lys Ile Phe Pro Gly Val
    130                 135                 140
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Thr Gln Tyr Glu Val Leu Gln Asn Val Cys Gly Asn Ile Arg Asp Phe
145 150 155 160

His Gly Leu Ile Leu Asn Gly Cys Ile Lys His Glu Met Val Glu Asn
165 170 175

Val Leu Pro Leu Phe Gln Leu Met Ala Glu Arg Val Gly His Phe Leu
180 185 190

Trp Glu Asp Gln Thr Asp Glu Asp Ser Arg Leu Ser Glu Leu Asp Glu
195 200 205

Asp Glu His Asn Asp Arg Asp Ser Arg Leu Phe Gln Leu Thr His Leu
210 215 220

Leu Leu Lys Ile Val Pro Thr Glu Leu Glu Val Met His Ile Cys Tyr
225 230 235 240

Thr Asn Leu Lys Ala Ser Thr Ser Ala Glu Val Gly Arg Phe Ile Lys
245 250 255

Lys Leu Leu Glu Thr Ser Pro Asp Ile Leu Arg Glu Tyr Ile Ile Gln
260 265 270

Leu Gln Glu His Met Leu Thr Val Ile Pro Pro Ser Thr Leu Gly Ala
275 280 285

Arg Asn Ile His Val Met Met Glu Phe Leu Leu Leu Ile Leu Ser Asp
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Met Pro Lys Asp Phe Ile His His Asp Lys Leu Phe Asp Leu Leu Ala
305 310 315 320

His Val Gly Thr Leu Thr Arg Glu Val Ser Thr Leu Val Arg Asp Leu
325 330 335

Glu Glu Lys Leu Arg Asn Lys Glu Gly Asn Asn Gln Thr Asn Cys Ala
340 345 350

Thr Leu Asp Leu Leu Glu Asn Ile Glu Leu Leu Lys Lys Asp Leu Lys
355 360 365

His Val Tyr Leu Lys Ala Pro Asn Ser Ser Gln Cys Cys Phe Pro Met
370 375 380

Ser Asp Gly Pro Leu Phe Met His Leu Leu His Met His Leu Asn Asp
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Leu Leu Asp Ser Asn Ala Tyr Ser Ile Ser Leu Ile Lys Glu Glu Ile
405 410 415

Glu Leu Val Ser Gln Glu Leu Glu Phe Ile Arg Ser Phe Phe Gly Asp
420 425 430

Ala Ala Glu Gln Gly Leu Tyr Lys Asp Ile Trp Ala Arg Val Leu Asp
435 440 445

Val Ala Tyr Glu Ala Lys Asp Val Ile Asp Ser Ile Ile Val Arg Asp
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Asn Gly Leu Leu His Leu Ile Phe Ser Leu Pro Ile Thr Ile Lys Lys
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Ile Lys Leu Ile Lys Glu Glu Ile Ser Ala Leu Asp Glu Asn Ile Pro
485 490 495

Lys Asp Arg Gly Leu Ile Val Val Asn Ser Pro Lys Lys Pro Val Glu
500 505 510

Arg Lys Ser Leu Thr Thr Asp Lys Ile Ile Val Gly Phe Glu Glu Glu
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Val Ile Ser Ile Thr Gly Met Pro Gly Ser Gly Lys Thr Thr Leu Ala
545 550 555 560

Tyr Lys Val Tyr Asn Asp Lys Ser Val Ser Arg His Phe Asp Leu Arg
565 570 575

Ala Trp Cys Thr Val Asp Gln Gly Tyr Asp Asp Lys Lys Leu Leu Asp
580 585 590

Thr Ile Phe Ser Gln Val Ser Gly Ser Asp Ser Asn Leu Ser Glu Asn
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Ile Asp Val Ala Asp Lys Leu Arg Lys Gln Leu Phe Gly Lys Arg Tyr
610 615 620

Leu Ile Val Leu Asp Asp Val Trp Asp Thr Thr Thr Leu Asp Glu Leu
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Thr Arg Pro Phe Pro Glu Ala Lys Lys Gly Ser Arg Ile Ile Leu Thr
645 650 655

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Leu Asp Leu Arg Leu Leu Arg Pro Asp Glu Ser Trp Glu Leu Leu Asp
675 680 685

Lys Arg Thr Phe Gly Asn Glu Ser Cys Pro Asp Glu Leu Leu Asp Val
690 695 700

Gly Lys Glu Ile Ala Glu Asn Cys Lys Gly Leu Pro Leu Val Ala Asp
705 710 715 720

Leu Ile Ala Gly Val Ile Ala Gly Arg Glu Lys Lys Arg Ser Val Trp

725 730 735

Leu Glu Val Gln Ser Ser Leu Ser Ser Phe Ile Leu Asn Ser Glu Val
740 745 750

Glu Val Met Lys Val Ile Glu Leu Ser Tyr Asp His Leu Pro His His
755 760 765

Leu Lys Pro Cys Leu Leu His Phe Ala Ser Trp Pro Lys Asp Thr Pro
770 775 780

Leu Thr Ile Tyr Leu Phe Thr Val Tyr Leu Gly Ala Glu Gly Phe Val
785 790 795 800

Glu Lys Thr Glu Met Lys Gly Ile Glu Glu Val Val Lys Ile Tyr Met
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Asp Asp Leu Ile Ser Ser Ser Leu Val Ile Cys Phe Asn Glu Ile Gly
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Asp Ile Leu Asn Phe Gln Ile His Asp Leu Val His Asp Phe Cys Leu
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Ile Lys Ala Arg Lys Glu Asn Leu Phe Asp Arg Ile Arg Ser Ser Ala
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Pro Ser Asp Leu Leu Pro Arg Gln Ile Thr Ile Asp Tyr Asp Glu Glu
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Glu Glu His Phe Gly Leu Asn Phe Val Met Phe Asp Ser Asn Lys Lys
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Arg His Ser Gly Lys His Leu Tyr Ser Leu Arg Ile Asn Gly Asp Gln
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Leu Asp Asp Ser Val Ser Asp Ala Phe His Leu Arg His Leu Arg Leu
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Ile Arg Val Leu Asp Leu Glu Pro Ser Leu Ile Met Val Asn Asp Ser
930 935 940

Leu Leu Asn Glu Ile Cys Met Leu Asn His Leu Arg Tyr Leu Arg Ile
945 950 955 960

Arg Thr Gln Val Lys Tyr Leu Pro Phe Ser Phe Ser Asn Leu Trp Asn
965 970 975

Leu Glu Ser Leu Phe Val Ser Asn Lys Gly Ser Ile Leu Val Leu Leu
980 985 990

Pro Arg Ile Leu Asp Leu Val Lys Leu Arg Val Leu Ser Val Gly Ala
995 1000 1005

Cys Ser Phe Phe Asp Met Asp Ala Asp Glu Ser Ile Leu Ile Ala
1010 1015 1020

Lys Asp Thr Lys Leu Glu Asn Leu Arg Ile Leu Gly Glu Leu Leu
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Ile Ser Tyr Ser Lys Asp Thr Met Asn Ile Phe Lys Arg Phe Pro
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Asn Leu Gln Val Leu Gln Phe Glu Leu Lys Glu Ser Trp Asp Tyr
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Ser Thr Glu Gln His Trp Phe Pro Lys Leu Asp Cys Leu Thr Glu
1070 1075 1080

Leu Glu Thr Leu Cys Val Gly Phe Lys Ser Ser Asn Thr Asn His
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Cys Gly Ser Ser Val Val Thr Asn Arg Pro Trp Asp Phe His Phe
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Pro Ser Asn Leu Lys Glu Leu Leu Leu Tyr Asp Phe Pro Leu Thr
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Asn Leu Glu Lys Leu Lys Leu Gln Glu Cys Gly Lys Leu Glu Glu
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Ile Pro Pro Ser Phe Gly Asp Ile Tyr Ser Leu Lys Phe Ile Lys
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Ile Val Lys Ser Pro Gln Leu Glu Asp Ser Ala Leu Lys Ile Lys
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<223> Rpi-blb or RB (Song, PNAS, 2003, 9128-9133)

<400> 93

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Met Ala Glu Ala Phe Ile Gln Val Leu Leu Asp Asn Leu Thr Ser Phe
1               5                  10                  15

ctc aaa ggg gaa ctt gta ttg ctt ttc ggt ttt caa gat gag ttc caa      96
Leu Lys Gly Glu Leu Val Leu Leu Phe Gly Phe Gln Asp Glu Phe Gln
            20                  25                  30

agg ctt tca agc atg ttt tct aca att caa gcc gtc ctt gaa gat gct     144
Arg Leu Ser Ser Met Phe Ser Thr Ile Gln Ala Val Leu Glu Asp Ala
        35                  40                  45

cag gag aag caa ctc aac aac aag cct cta gaa aat tgg ttg caa aaa     192
Gln Glu Lys Gln Leu Asn Asn Lys Pro Leu Glu Asn Trp Leu Gln Lys
    50                  55                  60

ctc aat gct gct aca tat gaa gtc gat gac atc ttg gat gaa tat aaa     240
Leu Asn Ala Ala Thr Tyr Glu Val Asp Asp Ile Leu Asp Glu Tyr Lys
65                  70                  75                  80

acc aag gcc aca aga ttc tcc cag tct gaa tat ggc cgt tat cat cca     288
Thr Lys Ala Thr Arg Phe Ser Gln Ser Glu Tyr Gly Arg Tyr His Pro
                85                  90                  95

aag gtt atc cct ttc cgt cac aag gtc ggg aaa agg atg gac caa gtg     336
Lys Val Ile Pro Phe Arg His Lys Val Gly Lys Arg Met Asp Gln Val
            100                 105                 110

atg aaa aaa cta aag gca att gct gag gaa aga aag aat ttt cat ttg     384
Met Lys Lys Leu Lys Ala Ile Ala Glu Glu Arg Lys Asn Phe His Leu
        115                 120                 125

cac gaa aaa att gta gag aga caa gct gtt aga cgg gaa aca ggt tct     432
His Glu Lys Ile Val Glu Arg Gln Ala Val Arg Arg Glu Thr Gly Ser
    130                 135                 140

gta tta acc gaa ccg cag gtt tat gga aga gac aaa gag aaa gat gag     480
Val Leu Thr Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp Glu
145                 150                 155                 160

ata gtg aaa atc cta ata aac aat gtt agt gat gcc caa cac ctt tca     528
Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asp Ala Gln His Leu Ser
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                    165                 170                 175
gtc ctc cca ata ctt ggt atg ggg gga tta gga aaa acg act ctt gcc      576
Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala
            180                 185                 190

caa atg gtc ttc aat gac cag aga gtt act gag cat ttc cat tcc aaa      624
Gln Met Val Phe Asn Asp Gln Arg Val Thr Glu His Phe His Ser Lys
        195                 200                 205

ata tgg att tgt gtc tcg gaa gat ttt gat gag aag agg tta ata aag      672
Ile Trp Ile Cys Val Ser Glu Asp Phe Asp Glu Lys Arg Leu Ile Lys
    210                 215                 220

gca att gta gaa tct att gaa gga agg cca cta ctt ggt gag atg gac      720
Ala Ile Val Glu Ser Ile Glu Gly Arg Pro Leu Leu Gly Glu Met Asp
225                 230                 235                 240

ttg gct cca ctt caa aag aag ctt cag gag ttg ctg aat gga aaa aga      768
Leu Ala Pro Leu Gln Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg
                245                 250                 255

tac ttg ctt gtc tta gat gat gtt tgg aat gaa gat caa cag aag tgg      816
Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Gln Lys Trp
            260                 265                 270

gct aat tta aga gca gtc ttg aag gtt gga gca agt ggt gct tct gtt      864
Ala Asn Leu Arg Ala Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val
        275                 280                 285

cta acc act act cgt ctt gaa aag gtt gga tca att atg gga aca ttg      912
Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu
    290                 295                 300

caa cca tat gaa ctg tca aat ctg tct caa gaa gat tgt tgg ttg ttg      960
Gln Pro Tyr Glu Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu
305                 310                 315                 320

ttc atg caa cgt gca ttt gga cac caa gaa gaa ata aat cca aac ctt     1008
Phe Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Pro Asn Leu
                325                 330                 335

gtg gca atc gga aag gag att gtg aaa aaa agt ggt ggt gtg cct cta     1056
Val Ala Ile Gly Lys Glu Ile Val Lys Lys Ser Gly Gly Val Pro Leu
            340                 345                 350

gca gcc aaa act ctt gga ggt att ttg tgc ttc aag aga gaa gaa aga     1104
Ala Ala Lys Thr Leu Gly Gly Ile Leu Cys Phe Lys Arg Glu Glu Arg
        355                 360                 365

gca tgg gaa cat gtg aga gac agt ccg att tgg aat ttg cct caa gat     1152
Ala Trp Glu His Val Arg Asp Ser Pro Ile Trp Asn Leu Pro Gln Asp
    370                 375                 380

gaa agt tct att ctg cct gcc ctg agg ctt agt tac cat caa ctt cca     1200
Glu Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser Tyr His Gln Leu Pro
385                 390                 395                 400

ctt gat ttg aaa caa tgc ttt gcg tat tgt gcg gtg ttc cca aag gat     1248
Leu Asp Leu Lys Gln Cys Phe Ala Tyr Cys Ala Val Phe Pro Lys Asp
                405                 410                 415

gcc aaa atg gaa aaa gaa aag cta atc tct ctc tgg atg gcg cat ggt     1296
Ala Lys Met Glu Lys Glu Lys Leu Ile Ser Leu Trp Met Ala His Gly
            420                 425                 430

ttt ctt tta tca aaa gga aac atg gag cta gag gat gtg ggc gat gaa     1344
Phe Leu Leu Ser Lys Gly Asn Met Glu Leu Glu Asp Val Gly Asp Glu
        435                 440                 445

gta tgg aaa gaa tta tac ttg agg tct ttt ttc caa gag att gaa gtt     1392
Val Trp Lys Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val
    450                 455                 460
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aaa gat ggt aaa act tat ttc aag atg cat gat ctc atc cat gat ttg 1440
Lys Asp Gly Lys Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu
465 470 475 480

gca aca tct ctg ttt tca gca aac aca tca agc agc aat atc cgt gaa 1488
Ala Thr Ser Leu Phe Ser Ala Asn Thr Ser Ser Ser Asn Ile Arg Glu
485 490 495

ata aat aaa cac agt tac aca cat atg atg tcc att ggt ttc gcc gaa 1536
Ile Asn Lys His Ser Tyr Thr His Met Met Ser Ile Gly Phe Ala Glu
500 505 510

gtg gtg ttt ttt tac act ctt ccc ccc ttg gaa aag ttt atc tcg tta 1584
Val Val Phe Phe Tyr Thr Leu Pro Pro Leu Glu Lys Phe Ile Ser Leu
515 520 525

aga gtg ctt aat cta ggt gat tcg aca ttt aat aag tta cca tct tcc 1632
Arg Val Leu Asn Leu Gly Asp Ser Thr Phe Asn Lys Leu Pro Ser Ser
530 535 540

att gga gat cta gta cat tta aga tac ttg aac ctg tat ggc agt ggc 1680
Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Tyr Gly Ser Gly
545 550 555 560

atg cgt agt ctt cca aag cag tta tgc aag ctt caa aat ctg caa act 1728
Met Arg Ser Leu Pro Lys Gln Leu Cys Lys Leu Gln Asn Leu Gln Thr
565 570 575

ctt gat cta caa tat tgc acc aag ctt tgt tgt ttg cca aaa gaa aca 1776
Leu Asp Leu Gln Tyr Cys Thr Lys Leu Cys Cys Leu Pro Lys Glu Thr
580 585 590

agt aaa ctt ggt agt ctc cga aat ctt tta ctt gat ggt agc cag tca 1824
Ser Lys Leu Gly Ser Leu Arg Asn Leu Leu Leu Asp Gly Ser Gln Ser
595 600 605

ttg act tgt atg cca cca agg ata gga tca ttg aca tgc ctt aag act 1872
Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys Thr
610 615 620

cta ggt caa ttt gtt gtt gga agg aag aaa ggt tat caa ctt ggt gaa 1920
Leu Gly Gln Phe Val Val Gly Arg Lys Lys Gly Tyr Gln Leu Gly Glu
625 630 635 640

cta gga aac cta aat ctc tat ggc tca att aaa atc tcg cat ctt gag 1968
Leu Gly Asn Leu Asn Leu Tyr Gly Ser Ile Lys Ile Ser His Leu Glu
645 650 655

aga gtg aag aat gat aag gac gca aaa gaa gcc aat tta tct gca aaa 2016
Arg Val Lys Asn Asp Lys Asp Ala Lys Glu Ala Asn Leu Ser Ala Lys
660 665 670

ggg aat ctg cat tct tta agc atg agt tgg aat aac ttt gga cca cat 2064
Gly Asn Leu His Ser Leu Ser Met Ser Trp Asn Asn Phe Gly Pro His
675 680 685

ata tat gaa tca gaa gaa gtt aaa gtg ctt gaa gcc ctc aaa cca cac 2112
Ile Tyr Glu Ser Glu Glu Val Lys Val Leu Glu Ala Leu Lys Pro His
690 695 700

tcc aat ctg act tct tta aaa atc tat ggc ttc aga gga atc cat ctc 2160
Ser Asn Leu Thr Ser Leu Lys Ile Tyr Gly Phe Arg Gly Ile His Leu
705 710 715 720

cca gag tgg atg aat cac tca gta ttg aaa aat att gtc tct att cta 2208
Pro Glu Trp Met Asn His Ser Val Leu Lys Asn Ile Val Ser Ile Leu
725 730 735

att agc aac ttc aga aac tgc tca tgc tta cca ccc ttt ggt gat ctg 2256
Ile Ser Asn Phe Arg Asn Cys Ser Cys Leu Pro Pro Phe Gly Asp Leu
740 745 750

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cct tgt cta gaa agt cta gag tta cac tgg ggg tct gcg gat gtg gag      2304
Pro Cys Leu Glu Ser Leu Glu Leu His Trp Gly Ser Ala Asp Val Glu
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tat gtt gaa gaa gtg gat att gat gtt cat tct gga ttc ccc aca aga      2352
Tyr Val Glu Glu Val Asp Ile Asp Val His Ser Gly Phe Pro Thr Arg
    770                 775                 780

ata agg ttt cca tcc ttg agg aaa ctt gat ata tgg gac ttt ggt agt      2400
Ile Arg Phe Pro Ser Leu Arg Lys Leu Asp Ile Trp Asp Phe Gly Ser
785                 790                 795                 800

ctg aaa gga ttg ctg aaa aag gaa gga gaa gag caa ttc cct gtg ctt      2448
Leu Lys Gly Leu Leu Lys Lys Glu Gly Glu Glu Gln Phe Pro Val Leu
                805                 810                 815

gaa gag atg ata att cac gag tgc cct ttt ctg acc ctt tct tct aat      2496
Glu Glu Met Ile Ile His Glu Cys Pro Phe Leu Thr Leu Ser Ser Asn
            820                 825                 830

ctt agg gct ctt act tcc ctc aga att tgc tat aat aaa gta gct act      2544
Leu Arg Ala Leu Thr Ser Leu Arg Ile Cys Tyr Asn Lys Val Ala Thr
        835                 840                 845

tca ttc cca gaa gag atg ttc aaa aac ctt gca aat ctc aaa tac ttg      2592
Ser Phe Pro Glu Glu Met Phe Lys Asn Leu Ala Asn Leu Lys Tyr Leu
    850                 855                 860

aca atc tct cgg tgc aat aat ctc aaa gag ctg cct acc agc ttg gct      2640
Thr Ile Ser Arg Cys Asn Asn Leu Lys Glu Leu Pro Thr Ser Leu Ala
865                 870                 875                 880

agt ctg aat gct ttg aaa agt cta aaa att caa ttg tgt tgc gca cta      2688
Ser Leu Asn Ala Leu Lys Ser Leu Lys Ile Gln Leu Cys Cys Ala Leu
                885                 890                 895

gag agt ctc cct gag gaa ggg ctg gaa ggt tta tct tca ctc aca gag      2736
Glu Ser Leu Pro Glu Glu Gly Leu Glu Gly Leu Ser Ser Leu Thr Glu
            900                 905                 910

tta ttt gtt gaa cac tgt aac atg cta aaa tgt tta cca gag gga ttg      2784
Leu Phe Val Glu His Cys Asn Met Leu Lys Cys Leu Pro Glu Gly Leu
        915                 920                 925

cag cac cta aca acc ctc aca agt tta aaa att cgg gga tgt cca caa      2832
Gln His Leu Thr Thr Leu Thr Ser Leu Lys Ile Arg Gly Cys Pro Gln
    930                 935                 940

ctg atc aag cgg tgt gag aag gga ata gga gaa gac tgg cac aaa att      2880
Leu Ile Lys Arg Cys Glu Lys Gly Ile Gly Glu Asp Trp His Lys Ile
945                 950                 955                 960

tct cac att cct aat gtg aat ata tat att taa                          2913
Ser His Ile Pro Asn Val Asn Ile Tyr Ile
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20 25 30

Arg Leu Ser Ser Met Phe Ser Thr Ile Gln Ala Val Leu Glu Asp Ala
35 40 45

Gln Glu Lys Gln Leu Asn Asn Lys Pro Leu Glu Asn Trp Leu Gln Lys
50 55 60

Leu Asn Ala Ala Thr Tyr Glu Val Asp Asp Ile Leu Asp Glu Tyr Lys
65 70 75 80

Thr Lys Ala Thr Arg Phe Ser Gln Ser Glu Tyr Gly Arg Tyr His Pro
85 90 95

Lys Val Ile Pro Phe Arg His Lys Val Gly Lys Arg Met Asp Gln Val
100 105 110

Met Lys Lys Leu Lys Ala Ile Ala Glu Glu Arg Lys Asn Phe His Leu
115 120 125

His Glu Lys Ile Val Glu Arg Gln Ala Val Arg Arg Glu Thr Gly Ser
130 135 140

Val Leu Thr Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp Glu
145 150 155 160

Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asp Ala Gln His Leu Ser
165 170 175

Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala
180 185 190

Gln Met Val Phe Asn Asp Gln Arg Val Thr Glu His Phe His Ser Lys
195 200 205

Ile Trp Ile Cys Val Ser Glu Asp Phe Asp Glu Lys Arg Leu Ile Lys
210 215 220

Ala Ile Val Glu Ser Ile Glu Gly Arg Pro Leu Leu Gly Glu Met Asp
225 230 235 240

Leu Ala Pro Leu Gln Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg
245 250 255

Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Gln Lys Trp
260 265 270

Ala Asn Leu Arg Ala Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val
275 280 285

Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu
290 295 300

Gln Pro Tyr Glu Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu

305 310 315 320

Phe Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Pro Asn Leu
325 330 335

Val Ala Ile Gly Lys Glu Ile Val Lys Lys Ser Gly Gly Val Pro Leu
340 345 350

Ala Ala Lys Thr Leu Gly Gly Ile Leu Cys Phe Lys Arg Glu Glu Arg
355 360 365

Ala Trp Glu His Val Arg Asp Ser Pro Ile Trp Asn Leu Pro Gln Asp
370 375 380

Glu Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser Tyr His Gln Leu Pro
385 390 395 400

Leu Asp Leu Lys Gln Cys Phe Ala Tyr Cys Ala Val Phe Pro Lys Asp
405 410 415

Ala Lys Met Glu Lys Glu Lys Leu Ile Ser Leu Trp Met Ala His Gly
420 425 430

Phe Leu Leu Ser Lys Gly Asn Met Glu Leu Glu Asp Val Gly Asp Glu
435 440 445

Val Trp Lys Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val
450 455 460

Lys Asp Gly Lys Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu
465 470 475 480

Ala Thr Ser Leu Phe Ser Ala Asn Thr Ser Ser Ser Asn Ile Arg Glu
485 490 495

Ile Asn Lys His Ser Tyr Thr His Met Met Ser Ile Gly Phe Ala Glu
500 505 510

Val Val Phe Phe Tyr Thr Leu Pro Pro Leu Glu Lys Phe Ile Ser Leu
515 520 525

Arg Val Leu Asn Leu Gly Asp Ser Thr Phe Asn Lys Leu Pro Ser Ser
530 535 540

Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Tyr Gly Ser Gly
545 550 555 560

Met Arg Ser Leu Pro Lys Gln Leu Cys Lys Leu Gln Asn Leu Gln Thr
565 570 575

Leu Asp Leu Gln Tyr Cys Thr Lys Leu Cys Cys Leu Pro Lys Glu Thr
580 585 590

Ser Lys Leu Gly Ser Leu Arg Asn Leu Leu Leu Asp Gly Ser Gln Ser
595 600 605

Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys Thr
610 615 620

Leu Gly Gln Phe Val Val Gly Arg Lys Lys Gly Tyr Gln Leu Gly Glu
625 630 635 640

Leu Gly Asn Leu Asn Leu Tyr Gly Ser Ile Lys Ile Ser His Leu Glu
645 650 655

Arg Val Lys Asn Asp Lys Asp Ala Lys Glu Ala Asn Leu Ser Ala Lys
660 665 670

Gly Asn Leu His Ser Leu Ser Met Ser Trp Asn Asn Phe Gly Pro His
675 680 685

Ile Tyr Glu Ser Glu Glu Val Lys Val Leu Glu Ala Leu Lys Pro His
690 695 700

Ser Asn Leu Thr Ser Leu Lys Ile Tyr Gly Phe Arg Gly Ile His Leu
705 710 715 720

Pro Glu Trp Met Asn His Ser Val Leu Lys Asn Ile Val Ser Ile Leu
725 730 735

Ile Ser Asn Phe Arg Asn Cys Ser Cys Leu Pro Pro Phe Gly Asp Leu
740 745 750

Pro Cys Leu Glu Ser Leu Glu Leu His Trp Gly Ser Ala Asp Val Glu
755 760 765

Tyr Val Glu Glu Val Asp Ile Asp Val His Ser Gly Phe Pro Thr Arg
770 775 780

Ile Arg Phe Pro Ser Leu Arg Lys Leu Asp Ile Trp Asp Phe Gly Ser
785 790 795 800

Leu Lys Gly Leu Leu Lys Lys Glu Gly Glu Glu Gln Phe Pro Val Leu
805 810 815

Glu Glu Met Ile Ile His Glu Cys Pro Phe Leu Thr Leu Ser Ser Asn
820 825 830

Leu Arg Ala Leu Thr Ser Leu Arg Ile Cys Tyr Asn Lys Val Ala Thr
835 840 845

Ser Phe Pro Glu Glu Met Phe Lys Asn Leu Ala Asn Leu Lys Tyr Leu
850 855 860

Thr Ile Ser Arg Cys Asn Asn Leu Lys Glu Leu Pro Thr Ser Leu Ala
865 870 875 880

Ser Leu Asn Ala Leu Lys Ser Leu Lys Ile Gln Leu Cys Cys Ala Leu
885 890 895

Glu Ser Leu Pro Glu Glu Gly Leu Glu Gly Leu Ser Ser Leu Thr Glu
900 905 910

Leu Phe Val Glu His Cys Asn Met Leu Lys Cys Leu Pro Glu Gly Leu
915 920 925

Gln His Leu Thr Thr Leu Thr Ser Leu Lys Ile Arg Gly Cys Pro Gln
930 935 940

Leu Ile Lys Arg Cys Glu Lys Gly Ile Gly Glu Asp Trp His Lys Ile
945 950 955 960

Ser His Ile Pro Asn Val Asn Ile Tyr Ile
965 970